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CONTENTS OF VOLUME 39, 1949

NO. 7. JULY

NO. 8. AUGUST

NO. 9. SEPTEMBER

ABSTRACTS OF PAPERS ACCEPTED FOR PRESENTATION AT THE FORTIETH ANNUAL MEETING OF THE SOCIETY, PITTSBURGH, PENNSYLVANIA DECEMBER 6 TO 8, 1948

A new glasshouse tomato variety, of Globe type, resistant to Fusarium oxysporum f. lycopersici race 1. ALEXANDER, L. J. In 1940 Bohn and Tucker described an immunity type of resistance to the Fusarium wilt disease of tomato. This resistance was inherited as a simple dominant factor. Several of the advanced generation lines of Bohn and Tucker were used as resistant parents in the present breeding project. Parents of one cross were Globe and line M-8-2, a resistant line of the following pedigree: Earliana × *Lycopersicon pimpinellifolium* (Missouri Accession 160) × Break O'Day × Ponderosa, field selfed, × Greater Baltimore, and field selfed twice. ''Ohio Wilt Resistant Globe'' resulted from crossing M 8-2 with Globe and backcrossing twice to Globe. The vine characteristics of Ohio W-R Globe closely resemble those of Globe, but the fruits tend to be slightly flatter and slightly smaller than those of Globe. The quality of the fruit equals or exceeds that of Globe. The yield of Ohio W-R Globe exceeded that of Globe in the spring crop of 1946, 1947, and 1948, and the fall crop of 1946. In 1945 Alexander and Tucker demonstrated the existence of physiologic races of the pathogen and described a new race. The distribution of the new race appeared to be limited to a small area in Ohio. Ohio W R Globe is susceptible to this race (2) of the pathogen.

Genetics of resistance to the common bean mosaic virus (bean virus 1) in the bean (Phaseolus vulgaris L.). ALI, MOHAMED A. K. Crosses were made between the susceptible variety Stringless Green Refugee and three resistant varieties, U.S. No. 5 Refugee, Idaho Refugee, and Robust, in all possible combinations. Results of inoculating F_2 plants indicate that resistance of U.S. No. 5 Refugee and Idaho Refugee is controlled by one dominant gene, while resistance of Robust is governed by a recessive gene. The results of reciprocal crosses were alike throughout. Robust × U.S. No. 5 Refugee gives in F_2 the ratio 13 resistant: 3 susceptible, suggesting a 2-gene difference. Using an approach-graft inoculation technique this ratio was broken down into 9 top necrotic : 4 resistant : 3 mosaic. Since top necrosis is characteristic of U.S. No. 5 and Idaho Refugee, but not of Robust, the results support the following factorial hypothesis: A basic dominant gene *A* is required for susceptibility, and another dominant gene *I* inhibits mosaic symptoms but gives top necrosis upon graft inoculation. Therefore, the genetic constitution of Stringless Green Refugee is *AAii* (susceptible), that of U.S. No. 5 Refugee and Idaho Refugee is *AAII* (dominant resistance and top necrotic reaction), and that of Robust is *aaii* (recessive resistance and no top necrosis). This explanation accounts for the two types of resistance and the distributions obtained from crosses between the varieties tested.

Rhizoctonia solani, a destructive pathogen of Alta fescue, smooth brome grass, and birdsfoot trefoil. ALLISON, J. LEWIS, HELEN S. SHERWIN, IAN FORBES, JR., AND ROBERT E. WAGNER. *Rhizoctonia solani* causes a disease of Alta fescue lawns and is a limiting factor in the establishment and persistence of smooth brome grass and birdsfoot trefoil in pasture mixtures at Beltsville, Maryland. During the summer seasons of 1947 and 1948, this fungus caused severe damage to spaced plants of fescue and brome grass in nurseries and to turf plots of these grasses in pure stand and mixtures. Trefoil in pure stand and mixtures was also severely attacked. A conspicuous leaf spot symptom was produced on the grasses, and infected plants were weakened and frequently killed outright. Surviving plants of the grasses and trefoil had some ability to recover. Damp, humid, warm weather favored disease development and dense, heavy vegetative growth was most susceptible to invasion by the fungus. The fungus mycelia grew rampant on all foliage parts and infection took place at random. Certain pasture grasses and legumes, orchard grass and Ladino clover particularly, had some resistance to natural infection with the fungus.

The Fusarium wilt of cowpeas and soybeans. ARMSTRONG, G. M., AND JOANNE K. ARMSTRONG. Observations of a few varieties of soybeans and numerous varieties or breeding lines of cowpeas in a field plot, as well as inoculation of these in the greenhouse with Fusarium-wilt isolates from both hosts, indicated that there are definitely two and probably other biological races on the basis of pathogenicity. Race 1 obtained from both soybeans and cowpeas caused wilting of some varieties of both hosts; while race 2 obtained only from cowpeas, caused severe wilting only of some varieties of this host. The cowpea varieties, Lady Finger and Sumptuous, served as differentials for separating races 1 and 2. Brown Sugar Crowder was susceptible, and Brabham and Iron generally were resistant to both races. No significant cultural or microscopic differences could be detected between the races, although the isolates represented a wide range of cultural types. Some isolates lost pathogenicity for soybeans but not for cowpeas. Inoculation experiments with one

1

or more isolates of both races 1 and 2 on the following plants failed to produce external symptoms of wilt: cotton, okra, coffeeweed, tobacco, sweet potato, mimosa, crotalaria, snapdragon, garden pea, pigeon pea, alfalfa, and hairy vetch.

Seed treatment tests with water-planted rice. ATKINS, J. G. JR., AND PAUL BOUCH-EREAU. In preliminary tests in 1948, rice was treated with several fungicides and planted in water. Three field tests were made in March and April. The slurry method was used, except with Yellow Cuprocide. Yellow Cuprocide gave an average increase of 53.8 per cent in emergence over nontreated seed. Emergence was not increased by Arasan SF (tetramethyl thiuram disulfide), the standard fungicide in Louisiana for drilled rice, or by Arasan SF plus two stickers, 31X and P.e.p.s. Dow 9B wettable (a trichlorophenate) drastically reduced emergence. Yield differences between nontreated, Yellow Cuprocide, and the Arasan SF treatments were small. The effectiveness of fungicides on pre-sprouted, water-planted rice was also studied, using pots filled with soil from rice fields. Yellow Cuprocide, red copper oxide, copper carbonate, and Tribasic copper sulphate increased emergence. In potted rice plants there was some inhibition of root development by the Cuprocide treatment. No inhibition was observed in field tests.

Western wheat mosaic in Colorado and its transmission by the grain aphid Toxoptera gramineum. ATKINSON, R. E. In 1947 an epidemic of wheat mosaic occurred in three wheat-growing districts of eastern Colorado. In 1948 the disease was generally less severe but was an important source of loss in two other districts. The virus nature of the disease was established by inoculating susceptible plants with filtered extracts of diseased plants. In repeated experiments plants grown in soil from infected fields have shown no mosaic symptoms. Experiments to ascertain if the virus is seed borne were negative. In the greenhouse, western wheat mosaic was transmitted by the grain aphid, *Toxoptera gramineum*. Single viruliferous aphids were isolated and implanted on pots of 10 healthy wheat plants. In every pot, 30 to 50 per cent of the plants had mosaic symptoms. Non-viruliferous aphids failed to produce symptoms of the mosaic although they were allowed to feed on the wheat until the plants died. The writer noted that plants affected with dry-land foot rot also had mosaic symptoms and extracts of these plants have uniformly produced mosaic in healthy potted plants. This suggests that a relationship exists between western wheat mosaic and dry-land foot rot in early planted wheat in Colorado.

Studies on the perithecial stage of Physalospora obtusa. AYCOCK, ROBERT. Perithecia of *Physalospora obtusa* occur abundantly in North Carolina on dead apple branches in the tree and on the ground. Periods of heavy ascospore discharge precede primary infections of apple leaves in the spring. These facts and other observations suggest that the sexual stage has a more important etiological role than is generally recognized. Cytological studies have revealed an unusual type of ascospore maturation. Following the third nuclear division in the young ascus 8 uninucleate spores are delimited by a process of free-cell formation. These young ascospores, which are about 3 μ in diameter, elongate until they measure about 3 by 24 μ. In unstained preparations they appear as long, filiform, intertwining strands. Subsequently, a marked increase in width occurs. Mature ascospores are ellipsoidal and average 13 by 30 μ. The young ascospores remain uninucleate until they are about 3 by 18 μ, after which several nuclear divisions occur prior to maturity, when as many as 16–20 nuclei have been observed. Comparisons of 24 single-ascospore cultures from 3 asci showed no clear-cut cultural or morphological differences.

Effects of excess solutes upon damping-off in sand cultures. BEACH, W. S. Using quartz sand in 8-inch stone jars as a medium, tomato seedlings were grown in six different strengths of Knop's solution, ½, 1, 2, 4, 8, and 16 × normal. Measures were taken to restore water lost by evaporation from the jars and to maintain pure cultures. At the time of planting (200 seeds per jar) the inoculum of a damping-off fungus was introduced in a circular pattern. It was found that Knop's solution, when added in concentrations stronger than 2 × normal, reduced the growth rates of both the seedlings and the fungi. There was more infection with *Rhizoctonia solani* and *Fusarium* as the 16 × normal solution was approached, however there was more infection with *Pythium ultimum* in the weaker solutions. *P. ultimum* did not grow in the 16 × normal solution, and seed inoculated with this fungus did not emerge at this concentration. Physiologic damping-off occurred with an abrupt rise of temperature and rapid concentration of the solutes.

Multiplication of clover club-leaf virus (Aureogenus clavifolium) in its insect vector. BLACK, L. M. A viruliferous female of *Agalliopsis novella* was mated with a non-viruliferous male. The pair was caged on a Grimm alfalfa plant (*Medicago sativa*)

because alfalfa is immune from the virus. The alfalfa was grown in soil that had been steamed to kill all weed seeds. Of the 42 progeny, 21 were tested individually on crimson clover (*Trifolium incarnatum*); 15 produced infections. Therefore, on the average, the virus in the original female had been diluted at least 1:30 among her progeny. The remaining 21 progeny were each placed on an alfalfa seedling and when they became adult, the females were mated to non-viruliferous males. The pair with the greatest number of progeny was chosen to continue the line. The experiment has been continued on alfalfa for 4 years through 15 generations without loss of infectivity. Non-viruliferous insects after feeding on the same alfalfa plants on which the 15 generations had been grown, failed to infect any of 275 clover plants, whereas, control insects inoculated 93 of 274 test plants after feeding on club-leaf clover. Assuming no multiplication, the estimated minimum dilution of the virus in the insects exceeded 10^{-17}. It is concluded that clover club leaf virus multiplies in its vector and is both an animal and a plant virus.

Comparative studies of a virus disease of cucumber transmitted mechanically from sour cherry and several cucumber virus diseases. BOYLE, J. S., J. DUAIN MOORE, AND G. W. KEITT. A virus disease transmitted mechanically to cucumber from young leaves of sour cherry showing necrotic ring spot symptoms was studied comparatively with several cucumber virus diseases. Inoculations were made on the cotyledons of young, potted cucumber plants held in a greenhouse air temperature range of 20° to 28° C. Symptom expression, host range, and certain property studies indicated characteristic differences between the cherry virus and the cucumber viruses used. On plants inoculated with the virus from cherry, the cotyledons persisted as yellow, mottled, turgid, and functional organs. The apical growing point was killed and about 30 to 45 days after inoculation bud proliferation with elongation occurred in the axis of the killed growing point with the subsequent development of a compact rosette of many flowers and dwarfed leaves. No herbaceous host other than cucumber has been found. The cherry virus had a thermal inactivation point of 52° C. for 10 minutes and a dilution end point of 1–20. Expressed juice kept at room temperature was infectious for only 12 hours. Final conclusions regarding the identity of the virus (or viruses) from cherry inciting the cucumber disease have not been reached.

Umbrella disease of Rhus glabra and R. typhina caused by Botryosphaeria ribis. BRAGONIER, WENDELL H. A disease resulting in cortical necrosis, wilting, and eventual death of plants of *Rhus glabra* L. and *R. typhina* Torn. is caused by the current cane blight organism, *Botryosphaeria ribis* G. and D. The disease present in Iowa, Minnesota, Missouri, Nebraska, and Wisconsin is responsible for widespread damage to roadside and natural plantings. In culture the pathogen produces abundant pycnidiospores on a wide variety of media under temperatures of 25° C. to 30° C. only if exposed to sunlight or the light from incandescent or fluorescent lamps.

Some effects of Fermate and Bordeaux sprays on Concord grapes. BRAUN, ALVIN J. Concord vines sprayed two seasons with Bordeaux mixture, 4–4–100 and 8–8–100, and with Fermate (ferric dimethyl dithiocarbamate), 1–100 and 2–100, showed accumulative effects of the treatments. The data were obtained from control-pruned vines on which the number of buds was determined by the weight of the previous season's shoot growth. Three applications of Bordeaux mixture decreased vine growth as measured by length of shoot and petiole. Similar applications of Fermate increased vine growth. Berry size was decreased by Bordeaux mixture and increased by Fermate. Bordeaux mixture delayed ripening of the fruit. The vines sprayed with Fermate yielded more than the unsprayed vines (yields adjusted for disease control) whereas those sprayed with Bordeaux mixture yielded less. By taking advantage of the increased vine growth resulting from the Fermate applications, greater increases in yields may be expected. Early applications of Fermate decreased the retarding effects of later applications of Bordeaux mixture.

Studies on variation in leaf rust of wheat, Puccinia triticina. BROWN, A. M. AND T. JOHNSON. In experiments designed to throw light on causes of pathogenic changes that have recently taken place in leaf rust of wheat no evidence was found to support the idea that pathogenic variation might proceed, in the uredial stage, through nuclear interchange between races. In the study of the possible role of *Thalictrum* spp. in originating new rust strains, greenhouse infection tests with native American species produced aecia on *T. dasycarpum*, pycnia only on *T. dioicum* and *T. polygamum*, and no infection on *T. venulosum*. Out-of-doors infection tests produced no infection on *T. dasycarpum* and *T. dioicum* but produced numerous aecia on the introduced *T. glaucum*. Selfing studies in which *Thalictrum* spp. were infected with races 3 and 5 indicated that the latter was homozygous whereas the former was heterozygous, pro-

ducing races 3, 15, 32, 68, and three undescribed races. A selfing of race 76 produced cultures with bright yellow urediospores that were low in virulence (resembling race 1), a new race with deep yellow uredia, and races 1, 29, and 101 with uredia of normal color.

A canker of Chinese elm. CARTER, J. C. Branch and trunk cankers were observed on several trees of *Ulmus parvifolia* Jacq. in November, 1946, in a nine-year-old planting at Urbana, Illinois. Several fungi were found fruiting in the cankerous bark. Cultures of the fungus which was isolated most consistently from the cankers were used to make eight inoculations in June, 1947. The technique of inoculation was to grow the fungus on sterile oat kernels, insert two or three fungus-bearing kernels beneath a triangular flap of bark on a leader, and seal the wound with Parafilm. This fungus and reisolations of it obtained from cankers which developed from the 1947 inoculations were used to make ten inoculations in May, 1948. All eighteen inoculations were followed by the development of cankers. The cankers resulting from the 1947 inoculations measured approximately 3.5 inches in length; those following the 1948 inoculations ranged from 3.75 to 9.5 inches in length. Infection became apparent eight to ten days after inoculation. Developing fruiting bodies of the fungus could be seen in about twenty days. The fungus has been classified tentatively as a *Tubercularia.*

Transmission of viruses through cherry seeds. CATION, DONALD. The Mahaleb and Montmorency seed collected from trees apparently infected with the cherry yellows virus complex were germinated in February and grown four months in the greenhouse. Grafts from the Mahaleb seedlings to seedling peach indicated over 20 per cent disease transmission with half of the infected peach trees showing ring spot and the other half showing typical cherry yellows symptoms. Similar inoculations from Montmorency seedlings resulted in over 20 per cent disease transmission, but only the ring spot virus was transmitted through Montmorency seed. Noninoculated peach trees of the same lot served as controls and grew normally without symptoms.

The constituents of "341" as apple scab fungicides. CHANDLER, W. A., AND H. W. THURSTON, JR. In 1948, the mixed glyoxalidine made from commercial stearic acid was formulated as the acetate salt dissolved in isopropanol (Experimental Fungicide 341–C). Commercial stearic acid contains approximately 45 per cent stearic, 45 per cent palmitic, and 10 per cent oleic acids. To test the relative effectiveness of these constituents of commercial stearic acid, glyoxalidines were prepared from synthetic stearic, palmitic, and oleic acids. These were formulated as the acetate salts in isopropanol and coded 341–SC, 341–PC, and 341–OC respectively. The 341–OC was very injurious to apple foliage and fruit, and it poorly controlled apple scab (*Venturia inaequalis* (Cooke) Winter). At ¼ lb. active glyoxalidine per 100 gallons, 341–C, 341–SC, and 341–PC controlled apple scab on susceptible McIntosh variety better than standard Lime Sulfur Flotation Sulfur. The 341–PC and 341–SC did not russet the fruit, and russet from 341–C was less than that from the standard schedule, confirming previous years' experience. The 341–C, 341–PC, and 341–SC in a lead arsenate, lime, nicotine insecticide schedule prevented build-up of European red mites so that special miticide sprays were unnecessary.

Mineral nutrition in relation to infection of the tomato by Septoria lycopersici. CHAPMAN, R. A. AND M. B. LINN. Tomato plants in quartz sand and solution cultures in the greenhouse were given purified nutrient solutions varying in the concentration of single nutrient elements. The plants were inoculated with *Septoria lycopersici* Speg. at the six leaf stage. Data for comparison were based on the number of lesions per unit of arbitrary leaf area of individual plants, obtained by summing the squares of the lengths of the leaves. Significant increases in amount of infection were obtained when nitrogen was increased from 21 to 630 p.p.m. and when boron was increased from 0.05 to 1.5 p.p.m. Significant decreases were obtained when manganese was increased from 0.05 to 1.5 p.p.m. and when copper was increased from 0.002 to 0.06 p.p.m. No significant differences were obtained when potassium was varied from 23.5 to 705 p.p.m., phosphorus from 3.1 to 93 p.p.m., iron from 0.1 to 3.0 p.p.m., and zinc from 0.005 to 0.15 p.p.m. Deficiency symptoms were obtained with the lowest levels of nitrogen, phosphorus, potassium, manganese, boron, and iron, but not with zinc and copper.

Soil treatments with chemicals for the control of tobacco parasites. CLAYTON, E. E., J. G. GAINES, T. W. GRAHAM, AND F. A. TODD. In the flue-cured tobacco growing area chemical soil treatments are effectively controlling both weeds and diseases. Plant bed treatments with calcium cyanamide and urea are well established. The following newer materials offer promise: Nitrites, especially calcium and potassium, at 1 lb. per square yard as weedicides, nematicides, and fungicides; sodium azide, ¼ lb. per square yard as a nematicide and fungicide; allyl alcohol, 6 qt. per 100 square yards as a weedicide.

Combinations of cyanamide and sodium azide, and allyl alcohol and ethylene dibromide, gave effective weed and root knot control. In field experiments D-D (dichloropropylene and dichloropropane) and ethylene dibromide gave good commercial control of root knot and nematode root rot (*Pratylenchus* spp.). Heavy reinfestation occurred by the end of the current crop year. The fumigation treatments did not affect the incidence of sore shin (*Rhizoctonia*), but in one experiment D-D gave excellent control of stem rot (*Sclerotium rolfsii*). With severe nematode infestation up to 50 per cent increase in the yield of cured leaf was obtained. In some experiments leaf quality was not affected, in others it was lowered.

Antibiosis of Actinomyces strains to Pythium arrhenomanes, P. ultimum, and Rhizoctonia solani. COOPER, W. E. AND S. J. P. CHILTON. A total of 2452 cultures of *Actinomyces* from 42 soil samples collected in Louisiana were tested for antibiosis on Czapek's agar to *Pythium arrhenomanes, P. ultimum,* and *Rhizoctonia solani.* There were 734 or 30 per cent of the cultures antibiotic to one or more of the three species of fungi. Of these, 588 were antibiotic to *P. arrhenomanes,* 335 were antibiotic to *P. ultimum,* and 345 to *R. solani.* More cultures showed a higher antibiosis to *P. arrhenomanes* than to the other two species. Cultures of *Actinomyces* were found which were antibiotic to one of the above three fungi, to all three of them, and to any two of them. At least three and probably more antibiotic substances were produced by the *Actinomyces* cultures tested. The antibiosis of a culture to one species was no index of its antibiosis to the other two species. No correlation was observed between soil type and the occurrence of strains antibiotic to *P. ultimum* and *R. solani.* The results indicate that in an analysis of the antibiotic potential of the *Actinomyces* present in a soil the cultures must be tested against a specific fungus.

Further studies on stem anthracnose of Lima bean in North Carolina. COX, ROBERT S. *Colletotrichum truncatum* is pathogenic on species from 11 genera of the Leguminoseae, and it can overwinter on exposed Lima bean refuse. Its cardinal temperatures for growth in culture were 5°, 27°, and 35° C. On inoculated Lima bean leaves the spores germinate, produce appressoria from which infection hyphae penetrate the cuticle and form primary hyphae in and between epidermal cells, within 22 hours. Within 46 hours subepidermal cells are penetrated and contents collapsed. A vascular discoloration as far as 150 mm. from the point of inoculation is evident within 5 days after injecting spore suspensions into stems and petioles with a hypodermic needle. Demonstration of the fungus in the xylem elements in free hand sections and other evidence suggests that spores are transported in the xylem. In 1948 the average yields (bu.) per acre of marketable green pods from nontreated plots and from those sprayed with the naphthoquinone Phygon XL (0.5 , 1.0 , and 1.5 100), or with the dithiocarbamates Dithane Z-78 (1.5-100), Zerlate (1.5 100), and Fermate (3.0 100) were 191, 317, 297, 283, 355, 296, and 282, respectively; whereas, the percentages of spotted pods were 58, 57, 44, 33, 32, 58, and 55, respectively. Phygon XL was phytotoxic.

White tip of rice. CRALLEY, E. M. White tip, an important disease of rice in the United States, has heretofore been ascribed to mineral deficiencies in the soil. Recent investigations indicate that white tip is caused by a nematode. The symptoms of the disease vary considerably from those of a nematode disease caused by *Anguillulina angusta* (Butler) Goodey, described on rice in India but are very similar to a nematode disease, caused by *Aphelenchoides oryzae* Yokoo, observed by the writer on rice in Japan in 1947. The disease is seed borne. Viable nematodes have been found between the glumes and berries of infected rice seed 8 months after the rice was harvested. The actual survival period of the nematodes in infected seed is not known. The disease has been successfully controlled in the greenhouse by treating infected Arkrose rice seed with hot water. Hot-water seed treatment at 52°-53° C. for 15 minutes reduced infection on the growing plants from 75 per cent to less than 1 per cent. Thus far, in the United States, losses from the disease in the field have been reduced by early planting (April) and by growing resistant varieties such as Fortuna, Nira, and Bluebonnet.

Three years' tests with various compounds for control of bacterial spot of peach. DAINES, ROBERT H. During the past three years more than 20 compounds have been field-tested against bacterial spot (*Xanthomonas pruni*). Of the materials tested, zinc sulfate-lime, 8-12 lb. per 100 gallons; Delmo-Z-lime (a basic zinc sulfate), 1½-6; copper-8-quinolinolate-lime, 1-12; Tenn. copper "26"-lime-1-12; and an experimental compound gave some protection. Of the better known materials that provided little or no protection are Sulfur-6; four of the dithiocarbamates—Zerlate, 1 and 2; Fermate, 1½; Manganese ethylene bisdithiocarbamate, 1½; and Dithane Z-78, 1½; the naphthoquinone Phygon, ¼ and 1; and the glyoxylidine 341-B-1 gal. Although zinc sulfate-lime gave some protection against bacterial spot, severe foliage injury followed its

use in two of the last three years. During 1946, injury was confined to early spray applications. During 1948, however, foliage injury continued to develop during much of the season, and the crop on the zinc sulfate-lime sprayed trees was greatly reduced. Trees sprayed with several experimental compounds have consistently had more disease than nonsprayed trees. Where Gamtox was applied during the curculio period and water during the remainder of the season, as a coarse fog applied in a driving stream, bacterial spot was significantly increased as compared with the same treatment applied in a well-fogged spray at low pressure.

The phosphorus metabolism of three species of Helminthosporium as determined by use of radiophosphorus. DAVIDSON, R. S. The phosphorus metabolism of *Helmintho-sporium turcicum, H. maydis, H. carbonum* race I, and *H. carbonum* race II has been studied using radiophosphorus. Replicated single-spore cultures derived from isolates of the three species known to be pathogenic on corn were grown in a modified Czapek's nutrient solution, each culture having 0.025 mg. radiophosphorus initially available. The phosphorus content of the mycelial mats after ashing was determined by means of a Geiger-Muller counter. The average phosphorus content per mg. of ash after 90 days was as follows: *H. turcicum*, 2.07×10^{-6} gm.; *H. maydis*, 1.03×10^{-6} gm.; *H. carbonum* race I, 1.5×10^{-6} gm.; and *H. carbonum* race II, 0.97×10^{-6} gm. The average ash weight per culture for each species was 4.1, 6.4, 5.1, and 6.7 mg. respectively. One month following inoculation, individual spores of *H. turcicum* contained 3.0×10^{-11} gm. P, *H. maydis*, 3.4×10^{-11} gm., and *H. carbonum* race II, 0.7×10^{-11} gm. *H. turcicum* grown with 25, 10, 5, 2.5, 0.5, and 0.05 mg. of phosphorus per culture for 60 days utilized 4, 7, 12, 29, 88, and 86 per cent respectively, of the available phosphous.

Combating bean blight chemotherapeutically with benzoic acid and the salicylates. DIMOND, A. E. AND E. M. STODDARD. Systemic infections of *Xanthomonas phaseoli* on red kidney beans may be reduced significantly in incidence and severity through chemo-therapeutic treatment with salicylates and benzoic acid. Greenhouse grown plants in sand are fertilized with modified Hoagland's solution at a nutritional level for maximum susceptibility. They are systemically infected by pricking the pathogen into a young node of trifoliate leaves. Chemotherapeutic treatment consists of watering the plant with an aqueous solution of the test compound at non-phytotoxic concentration. Under these conditions, salicylic acid is effective at moderate but phytotoxic at high temperatures, acetyl salicylic acid is moderately effective, and 8-quinolinol salicylate is erratic. Phenyl and methyl salicylates are ineffective. In limited tests with salicylic acid, timing of applications with respect to inoculation and total number of applications have been varied. Treatment is apparently most effective when applications are made after inoculation. As the number of applications after inoculation is increased, the amount of protection is increased. To a much smaller extent, increasing number of applications before inoculation also increases protection. These results imply that salicylic acid is readily mobile, but rapidly metabolised in the plant.

On the genetics of resistance to mildew in barley. FAVRET, EWALD A. Studies were made at the University of California, on the inheritance of resistance to *Erysiphe graminis hordei* physiologic race 3, in several barley crosses. It was found that Monte Criste and Engledow India varieties carry a dominant gene for immunity, which has been called Ml^m and it is linked with one of the Nigrate factors determined by Briggs. The resistance of West China barley is induced by a single recessive factor, which has been called ml^w. It is probable that the resistance of Gopal barley was governed by two major genes, linked with a crossover value of about 15–20 per cent. With the two factors above mentioned, nine genes are already known to be involved in the resistance to mildew race 3 in barley, the largest number of loci related with resistance to a plant disease.

Physiological and chemotherapeutic investigations of Ceratostomella ulmi. FELDMAN, A. W., NESTOR E. CAROSELLI, AND FRANK L. HOWARD. To standardize laboratory biotitration techniques and toxin production, mycelial weight, oxygen tension, age, pH, acid production, reducing substances, and polysaccharide yield were studied in liquid shake cultures of *Ceratostomella ulmi* and were measured against wilt indices of sand-grown tomato cuttings. Highest toxin titre was obtained with sodium citrate–HCl buffered shake cultures (pH 4.25) in 7 days. No correlation was found between polysaccharide production in liquid culture and wilting. Culture extracts produced 100 per cent wilt when polysaccharide formation was eliminated by buffering. Toxin adjusted above pH 7 with Na or Ca(OH)₂ was inactivated. Cultures grown under high oxygen tension produced no wilt. By being predisposed to the dark for 5 days prior to inoculation, all two-year-old seedlings of *Ulmus americana* were diseased and had typical Dutch Elm symptoms. Of the 104 chemicals screened in the laboratory, 15 possessed some chemo-

therapeutic properties and so were further tested in the field as sprays, direct trunk injections, and soil injections under pressure. Three chemicals suppressed the disease symptoms during the season. Their effectiveness was greatly increased by soil liming. Weekly carbohydrate anaylses of the check trees and 3 treatments indicated a correlation between carbohydrate reserve and symptom incidence.

Copper naphthenate dust and spray. FELIX, E. L. Studies on copper naphthenate preparations safe to plants were initiated because of: (1) the well-known effectiveness of copper naphthenate against a variety of fungi in mildewproofing of cotton fabric, (2) its ability to withstand leaching, and (3) the results of limited tests in the field of agriculture. Dust was prepared from copper naphthenate dissolved in Stoddard solvent by mixing the following parts by weight, drying, and pulverizing: 70 per cent copper naphthenate 50, Attaclay 65 (3.5 per cent Cu); 80 per cent copper naphthenate 45, Attaclay 64 (3.6 per cent Cu); 80 per cent copper naphthenate 50, Attaclay 60 (4 per cent Cu). A dust of 35 per cent copper naphthenate in Attaclay (3.5 per cent Cu) possesses excellent flowability and adherence. Concentrations higher than 40 per cent are not useful in dust. The maximum concentration of metallic copper in naphthenate emulsion which can be used safely on some plants appears to be 0.0625 per cent, equivalent to 1-1-50 Bordeaux or less. The following emulsion (parts by volume) gives excellent wetting and an extremely fine spray: copper naphthenate 80 per cent in Stoddard solvent (Cuprinol Division) 50, Emulphor AG oil soluble 20, Emulphor ELA 10, mixed, and water 3720. Stock emulsions also may be prepared. Copper naphthenate in emulsion seems to penetrate quickly into plant tissues, leaving no visible residue and consequently minimum exposure to weathering. The value of copper naphthenate dusts and sprays in fungicidal crop protection and chemotherapy is being studied.

Host nutrition in relation to development of Verticillium wilt of tomato. GALLEGLY, M. E. Bonny Best tomato plants were grown in constant drip sand cultures of 0.1, 0.5, 1, 2, and 3 times a basal salt solution and in culture with the basal solution low and high in nitrogen, phosphorus, and potassium. The experiments were conducted during winter months with the greenhouse air temperature held at 20° C. and with fluorescent lamps regulated to give 12-hour days. Inoculations were made after 30 to 40 days' growth by dipping the washed roots in a concentrated mycelium and spore suspension of *Verticillium alboatrum*. In varying concentrations of the balanced solution, disease development was least at the 0.1 level and increased with an increase in salt concentration. In the unbalanced solution low in nitrogen, disease development was decreased; in the other solutions, disease development was no different from that in the basal solution.

Host nutrition and predisposition in relation to development of bacterial wilt of tomato. GALLEGLY, M. E. Development of wilt (*Pseudomonas solanacearum*) in plants grown in sand culture at nutrient concentrations 0.1, 0.5, 1, 2, and 3 times basal during summer months was greatest at 0.1 and decreased with an increase in salt concentration; during late autumn and early spring months disease development was greatest at 0.5 and 1. The first results were reproduced under controlled 18-hour day length while the second results were reproduced at 12-hour day lengths. Variation in light intensity and sand culture temperature failed to alter the long-day disease curve. In sand cultures with nutrients low or high in nitrogen, phosphorus, and potassium, disease development during summer months was retarded in the high nitrogen solution and increased in the low potassium solution; during early spring months both unbalanced nitrogen solutions reduced disease development. Pre-inoculation treatments including soil temperature, air temperature, day length, light intensity, and soil moistures had little effect on disease development. Post inoculation treatments showed maximum disease development to be favored by 36° C. soil temperature, 28° C. air temperature, short-day length, low light intensity, and medium and high soil moisture.

Inoculation experiments with Atlantic and Pacific Coast collections of Transschelia pruni-spinosae discolor. GOLDSWORTHY, M. C. AND JOHN C. DUNEGAN. Inoculation experiments with urediospores of *Transschelia pruni-spinosae discolor* from Fort Valley, Georgia and Yuba City, California were carried on in the greenhouses of the Plant Industry Station, Beltsville, Maryland during 1948. Leaf and twig infections were produced by both collections of the rust fungus on all 13 commercial peach varieties (5 from California and 8 from the Eastern Seaboard) tested. Leaves were susceptible as soon as they unfolded, with rust sori first appearing at the base of the blades. Bark infections however did not develop on the shoots until 6 to 8 leaves had formed, and the bark could still be infected 9 weeks after the shoot had started to develop. The Palora, a western variety, and Dixired and Dixigem, recently introduced eastern varieties, were the most susceptible of the 13 varieties tested in respect to bark infection, while Elberta

was the most resistant. These inoculation experiments indicate that the absence of bark lesions under natural conditions in eastern orchards, in contrast to their frequent occurrence in western orchards, is not due to a difference in the rust fungus in these two widely separated regions.

Influence of climate on incidence of Fusarium rot and dry rot in gladiolus corms. GOULD, CHARLES J. Fusarium rot (*Fusarium oxysporum* f. *gladioli*), the most troublesome disease of gladiolus corms in eastern and southern United States, is also severe in eastern Washington, but uncommon in western Washington except on recently introduced stocks. Dry rot (*Sclerotinia gladioli*) is the most important corm disease in the latter area. To study the role that climate plays in the development of these two diseases, corms of the variety Picardy were obtained from a stock that was severely infected with both diseases. Apparently healthy unhusked corms were selected from this stock and grown in the comparatively cool climate of western Washington at Puyallup, and in the warmer climate of central Washington at Sunnyside. At harvest, 76 per cent of the non-treated western Washington-grown corms had dry rot and 5 per cent had *Fusarium* rot. Comparable figures for the eastern Washington-grown corms were 17 per cent and 32 per cent, respectively. Temperature is believed to be the primary factor responsible for these differences. Average temperatures from May through September were 60° F. at Puyallup and 67° F. at Sunnyside. These results and previous observations indicate that rotation between different climatic areas might be a useful supplement to standard measures for controlling dry rot and fusarium rot in commercial gladiolus corms.

Phytotoxicity of certain fungicide treatments to narcissus bulbs. GOULD, C. J., W. D. MCCLELLAN, AND V. L. MILLER. Certain fungicide treatments applied to narcissus bulbs sometimes injure the bulbs and are followed by such distortion of the flowers that varietal identification is impossible. Bulbs treated with New Improved Ceresan (ethyl mercury phosphate), 2 per cent Ceresan (ethyl mercury chloride, or Ceresan M (ethyl mercury p toluene sulfonanilide) for the control of basal rot (*Fusarium oxysporum* f. *narcissi*) have produced the following abnormalities: perianth segments were paler than normal, proportionately narrower, and smaller; trumpet was paler, smaller, often lacked the typical recurved edge (King Alfred variety), and was frequently constricted around the exserted stigma and anthers; blasted flowers represented the extreme degree of flower injury; affected leaves were yellowish green and sometimes slightly swollen in the upper portion; severely injured bulbs decayed from the center outward. Injury most often occurred when bulbs were treated within three days after digging or when kept moist for several days after treating. Injuries produced by Dowicide B (sodium trichlorophenate) or by the warm water formaldehyde treatment for nematode control were distinctive. Flowers injured by Dowicide B failed to open normally and perianth segments were dwarfed and greenish-yellow. Bulbs treated at the wrong time with warm water and formaldehyde produced blasted or jagged distorted flowers and leaves mottled near the tips.

Nematode root rot of tobacco and other crops. GRAHAM, T. W. Nematode root rot caused by *Pratylenchus spp.*, is a typical brown root rot. It is a disease of economic importance throughout the Coastal Plain Region of southeastern United States. In South Carolina, with tobacco plants transplanted in early May, invasion of the roots begins a month later. Maximum nematode populations were found in the tobacco roots during late July and early August. By mid-August extensive root decay had occurred and coincidentally the number of nematodes in the roots rapidly declined. This decline was associated with a rapid build-up of nematodes in the roots of crab grass, a universal volunteer plant in tobacco fields during late summer and fall. Tobacco roots invaded by meadow nematodes break down and decay more rapidly than similarly invaded roots of corn, cotton, peanuts, or crab grass. Certain of these other plants, however, support larger nematode populations. In a field experiment set up to measure these differences, the comparative size of the nematode populations for equal weights of roots on August 5 was: tobacco, 1.0; cotton, 0.7; crab grass, 2.3; and corn, 18.7. Some of the grasses, and corn in particular, are preferred host plants for meadow nematodes. Tobacco grown after corn is often severely damaged by nematode root rot.

Inhibitory action of 2,4-Dichlorophenoxyacetic acid on Penicillium digitatum and Phomopsis citri. GUISCAFRÉ-ARRILLAGA, J. Among chemicals tested for prevention of post-harvest decay of oranges, 2,4-D showed strong inhibitory action against *Penicillium digitatum* and *Phomopsis citri*. Marked reduction in decay occurred in oranges dipped 3 to 5 minutes in 0.1 and 1.0 per cent solutions of two commercial preparations of sodium salt of 2,4-D (70 and 76 per cent acid equivalents). *P. digitatum* was the prevailing fungus. In other tests, conidial germination of *P. digitatum* and *P. citri* was

inhibited by the same dilutions of the two materials. A 75 per cent "micronized" 2,4-D acid was effective at 1:3000 against *P. digitatum* conidia and 1:10,000 against *P. citri* conidia. On Sabouraud's agar, the sodium salt (70 per cent acid equivalent) added at rates of 1:300, 1:500, and 1:1000 strongly inhibited growth, *P. citri* being more sensitive than *P. digitatum*. Orange peelings, dipped in conidial suspensions of these fungi, then in 1:100 to 1:1000 solutions of the sodium salt, and placed in moist chambers, remained free of fungus growth for 10 days. Checks were covered with mold. Some inhibition occurred against a strain of *Penicillium notatum* from the University of North Carolina.

Cross protection tests between the Wisconsin pea streak virus and several strains of bean virus 2 from pea. HAGEDORN, D. J. AND J. C. WALKER. Several strains of bean virus 2 were secured from pea plants affected with mosaic which had been sampled from 23 Wisconsin fields in 1948. Of these, 14 yielded viruses which were infective on Idaho Refugee bean and conformed to the characteristics incited by bean virus 2. The virus appears to be the most widespread on pea in Wisconsin. Wisconsin pea streak was prevalent in the latter part of the season, occurring often in conjunction with mosaic. Cross protection inoculations were set up to determine any possible affiliation between strains of bean virus 2 isolated from pea and the virus inciting Wisconsin pea streak. The latter developed readily in peas infected with the former. When soybean was inoculated with the streak virus there was no cross protection against bean virus 2 indicated. The two viruses thus may act additively in reducing pea yields.

Sex in Ustilago Lorentziana. HANNA, W. F. AND E. M. HURST. The four sporidia derived from a single chlamydospore of *Ustilago Lorentziana*, the fungus causing smut of wild barley (*Hordeum jubatum*) were cultured separately. When the monosporidial cultures were grown together in pairs on non-nutrient agar, fusions occurred between the sporidia of certain pairs of cultures only. Seedlings of *H. jubatum* inoculated with a pair of monosporidial cultures whose sporidia were capable of fusing with one another produced smutted plants, whereas those inoculated with other pairs of cultures or with a single monosporidial culture remained apparently uninfected. It is concluded that *U. Lorentziana* is heterothallic and bisexual.

Post-harvest chemical dip treatment of peaches for control of brown-rot disease in the package. HEUBERGER, J. W., G. D. MUNGER AND P. L. POULOS. Various water soluble fungicides were tested. Peaches were brushed, graded, dipped for 30 seconds, drained 3–5 minutes, packed in standard open-staved bushel baskets with liner and lid, and stored at room temperature 3 days. In exploratory experiments on the Halehaven variety it was found that Hyamine 1622 (a quaternary ammonium compound), Mycoban (sodium propionate), and Dithane D-14 (disodium ethylene bisdithiocarbamate) did not effect control at non-injurious concentrations but that Isothan Q-15 and Liquid Lime Sulfur did. In a definitive experiment on the Elberta variety, percentage rot data were as follows: water dip, 78.4; no treatment (absolute check), 52.3; Isothan Q-15 (2 qt.–100) 33.3, (1 pt.–100) 37.6; and Liquid Lime Sulfur (4 qt.–100) 19.6, (2 qt.–100) 20.3, (1 qt.–100) 28.6. In this same experiment, percentage rot data when packed bushel baskets were dipped were: Isothan Q-15 (1 pt.–100) 35.6 and Liquid Lime Sulphur (2 qt.–100) 16.8. When *Rhizopus* was present, Isothan Q-15 (lauryl isoquinolinium bromide) increased the amount of rot, Liquid Lime Sulfur effected no control, and storage at 42° F. gave perfect control. Isothan Q-15 did not affect the appearance, odor, or taste of the peaches; Liquid Lime Sulfur did not affect the appearance or taste, but did leave a slight sulfur odor which was distinguishable only at close range.

Relation of time of cutting to rate of decay of beech, birch, and spruce under natural storage conditions. HILBORN, M. T. AND F. H. STEINMETZ. That the time of cutting influences the rate of wood decay has been generally accepted for some time, but it has usually been stated that after 1 year's storage there is no difference in durability regardless of when the wood was cut. Data have been obtained for 3 species of wood cut at different times of the year and stored in the woods. Beech and white birch cut in March showed a higher rate of decay than when cut in July or September. White spruce cut in September showed more decay than when cut in March or July. These differences were apparent at the end of the first season, and continued to be expressed and intensified during the next 6 years. Removal of bark and splitting of larger bolts at the time of cutting considerably lessened the susceptibility to decay.

Location of bacteria in healthy potato tissue. HOLLIS, JOHN P. Isolatio potato seedpieces, stems, tubers and seed reveal the presence of a varied a

bacterial flora in the vascular tissues of all parts except seed. Isolates were obtained most readily from seedpieces and stems. The prevalence of bacteria in the seedpieces was in proportion to the degree of vascularization of the tissue. Many different types of bacteria were obtained from seedpieces. Only 3 types occurred repeatedly, but 14 were obtained more than once. Accordingly these 14 were considered as reference types. Isolates from stelar regions of stems and tubers of the same potato lot and from various seed lots were compared with these. Five of the 14 types were found also in stems, 3 in tubers, and none in seed. Seven of the 14 types were obtained from plants growing in both soil and peat. These results furnish evidence of the presence and location of a mixed, heterogeneous bacterial flora in the vascular elements of healthy potato plants and support the hypothesis that bacteria enter the plant through wounds made by the emergence of secondary roots. The mode of entry remains to be proven.

Serial mutations of tobacco-mosaic virus. HOLMES, FRANCIS O. Mutants of tobacco-mosaic virus, derived from each other in series, disclosed two characters that were strictly correlated, though other observed characters mutated independently. Low infectivity was associated invariably with inability of virus to move from the site of infection in tobacco and increased infectivity with change to ability to move systemically. Two non-systemic strains were studied in detail. One proved relatively stable, giving rise to no green-mottling mutants and to few yellow-mottling or necrotizing mutants. The other was less stable, giving rise to many green-mottling, but no other, types of derivatives. Systemically moving mutants were recovered without admixture of the parent strains, which had been used as original inocula, by subinoculation from newly developing leaves at the tops of the plants.

Bimodal dosage-response curves in fungicide field trials. HORSFALL, JAMES G., SAUL RICH, AND R. A. CHAPMAN. Fragments of bimodal dosage-response curves have resulted from field trials with several fungicides and several plant diseases. This is contrary to the expectation that effectiveness should decrease as dosage is decreased. Zinc ethylenebisdithiocarbamate (Z-78) sprayed on celery to control either *Septoria* or *Cercospora* yields a typical curve in which the protective action of the fungicide falls, rises, and falls again as the dosage decreases. The initial drop is due to the expected tendency of disease control to decrease with dosage. However, as Mason (1948) suggested, and as observed in our laboratory, as dosage decreases, a corresponding decrease takes place in the size of the aggregates of particles in the dried spray residue which expose a disproportionately greater specific surface area from which the toxicant can be dissolved. Increasing the specific surface area causes a higher rate of solution. The loss in toxicity due to decreasing dosage takes place more slowly than does the increase in toxicity due to increasing rate of solution. As both forces are concerned in the slope of the dosage-response curve, the rate of solution eventually dominates the downward trend due to loss in applied dosage, and the resultant is manifested as increasing toxicity. The final decline in toxicity is probably due to 1) further decline in dosage and 2) the effect described by Roller (1932) in which rate of solution falls off as decreasing particle size approaches the colloidal range.

Breeding for scab resistant apples. HOUGH, L. F., AND J. R. SHAY. In apple seedling orchards at Urbana, Illinois, and Lafayette, Indiana, 4664 scab-resistant apple seedlings have been established for fruiting tests. Of these, 871 are from crosses of 12 different small-fruited *Malus* species with Wolf River or Twenty Ounce, and 3793 are from crosses of scab-resistant selections with 2-inch fruits crossed with Jonathan, McIntosh, and Delicious. All progenies were grown from seeds in the greenhouse and scab-susceptible segregates were eliminated by artificial inoculations. Susceptible × *M. floribunda* gave a ratio of 1 resistant: 1 susceptible and *M. floribunda* selfed gave a ratio of 3:1. This indicates that scab resistance in this clone is dominant and controlled principally by a single factor pair for which *M. floribunda* is heterozygous. In other species, the inheritance is more complex but segregation in crosses with commercial apple varieties is distinct and ratios seem consistent.

Trapping sporangia of Phytophthora infestans as an aid in forecasting the development of late blight. HYRE, R. A. A series of eight spore traps was established from Virginia to Rhode Island. The traps consisted of 1 × 3 inch vaselined slides held in a vertical position in a vane and changed daily. In a period of about two months, 13 sporangia were trapped, with some question as to the identity of five of them. Late blight always occurred within 12 miles of the traps prior to the time the sporangia were caught. These data indicate that the traps were of little practical value for forecasting

the early occurrence of late blight in 1948. Trapping studies were also made with sporangia of *Pseudoperonospora cubensis* (B. and C.) to find a more efficient arrangement of the slide. In a 5-mile wind a significantly greater number of sporangia were trapped when the slide was placed in a horizontal position or into the wind on a 45-degree incline, with a shield in both cases, than when placed in a vertical position without a shield. The relative number of spores trapped follows: vertical position in vane, 1; horizontal position, 4.6; inclined 45 degrees, 5.5; slide in vertical position at exit of funnel 18 inches in diameter at entrance, 2.7.

Virus particles in vascular contents. JOHNSON, JAMES. By means of artificial water pressure, the vascular contents of virus diseased plants were obtained for electron microscopy. Materials of 24 virus diseases have been examined in this manner. Rod-like particles were found associated with 9, and spherical bodies apparently with 2 viruses. Cubicle crystals, not definitely associated with any virus, were occasionally observed. With 12 diseases, abnormal particles were apparently not regularly associated with the virus. Ordinary, yellow, or a mild strain of tobacco mosaic yielded typical rod-like particles in several solanaceous genera, including *Nicotiana, Lycopersicon, Physalis, Solanum, Petunia,* and *Capsicum;* and, in the non-solanaceous genera, *Digitalis, Eryngium,* and *Plantago.* Similar rod-like particles were obtained from potato-mottle, potato ring-spot, tobacco-streak, tobacco ring-spot, a garden pea mosaic, and cucumber virus 4. The failure to find virus particles by this method cannot yet be regarded as significant unless accompanied by inoculation tests showing the virus present in reasonable concentration in the vascular contents. The particles of the tobacco mosaic virus have been found in the vascular contents as early as 36 hours after inoculation. The variation of the virus particles in the vascular contents suggests the value of such material for studies on the origin of the particles.

The number of strains of the tobacco mosaic virus. JOHNSON, JAMES. Numerous single-lesion isolations of tobacco mosaic virus from a highly susceptible host were continued serially through many transfers. Various treatments were applied with the purpose of inducing variation. The tests for variation were largely based on symptom expression and behavior on tobacco (var. Havana). The age of the plants inoculated, the rate and vigor of plant growth, the temperature, and other conditions vary symptom expression greatly. Variations from such causes, especially when associated with strain mixtures, are likely to be misinterpreted as strain variations or mutations. Strains of indicated purity, as a result of multiple single-lesion transfers, have remained remarkably constant in successive transfers except when exposed to high temperatures, which may induce virus alteration. Variants rarely survive or are conspicuous in nature on tobacco, either because of competition with the ordinary tobacco mosaic virus, or because of the less favorable conditions for development on older plant tissues. About 20 distinguishable strains have been isolated from field tobacco mosaic. Most of these are similar to strains previously described. The belief that strains of the tobacco mosaic virus exist in much larger numbers, as a consequence of frequent mutation, does not appear to be justified.

Soil fumigation for cigar-wrapper tobacco in Florida. KINCAID, RANDALL R., AND GAYLORD M. VOLK. Triplicate plots were fumigated on each of three dates (Sept. 29 and Dec. 3, 1947, and Feb. 5, 1948) with each of two fumigants, Dowfume W-40 (ethylene dibromide), 15 gallons per acre, and D–D (dichloropropylene and dichloropropane), 23 gallons per acre, for comparison with nontreated checks. Plots fumigated in September were sowed in oats 12 days later. The growth of the tobacco crop transplanted on March 25, 1948, appeared much better with certain of the treatments than with no treatment. Control of root knot was best with the earliest application of each fumigant; D–D was slightly more effective in this experiment than Dowfume W–40. Control of coarse root (nematode root rot) was not so good as control of root knot. Following fumigation, there was a prolonged retention of ammonia nitrogen in the soil, extending into April with Dowfume W–40, and into May with D–D. The effects of fumigation on the ammonia level in the soil and consequently on the plant are entirely separate and apart from the control of nematodes, and should be differentiated for a proper understanding of fumigation.

Pathogenicity of Sclerotinia trifoliorum to some forage legumes. KREITLOW, K. W. Monoascospore isolates from apothecia of *Sclerotinia trifoliorum* on Ladino and red clover yielded several cultural types that varied from strictly mycelial forms to those that produced numerous sclerotia. Pathogenicity of monoascospore isolates was established on Ladino and red clover as well as on other species of legumes. Differences in

pathogenicity of each of four monoascospore isolates was determined with 60–70 plants of Ladino clover. Most species of *Trifolium, Medicago,* and *Melilotus* tested were susceptible to a composite sample of pathogenic isolates; several species of *Trifolium,* however, were resistant.

Winter injury versus disease in Wisconsin poplar plantings. KUNTZ, J. E., AND A. J. RIKER. Hybrid poplars have been reported to fail in the North Central Region primarily because of winter injury. However, the need in Wisconsin for such rapidly growing trees called for further experiments on selected sites. Thus, replicated plantings of cuttings from 33 hybrid poplars from the Northeastern Forest Experiment Station and from 6 local selections have been made at different times during the past seven years in eight widely scattered areas. Winter injury was encountered occasionally (1) with first season trees, (2) on the tips of succulent shoots, (3) in frost pockets, but was not a critical factor. Continued satisfactory growth was limited, however, by cankers that were usually associated with *Cytospora.* This was found fruiting abundantly and was consistently isolated from the most advanced margins of the cankers. *Septoria,* so important elsewhere, was observed and isolated only occasionally. Vigorous trees withstood the cankers best. These results indicate that the chief problem in Wisconsin is not one of winter injury but rather one of selection for disease resistance.

Reaction of potato varieties to the virulent ringspot virus. LADEBURG, R. C., AND R. H. LARSON. A single-lesion ringspot culture, isolated from a diseased Red Warba potato, was used as inoculum in testing 44 American and 4 British varieties, over a two-year period, in the greenhouse and in the field. Older American varieties, such as Cobbler, Triumph, Russet Rural, Green Mountain, Russet Burbank, White Rose, Early Ohio, etc., failed repeatedly to show symptoms when inoculated, while most of the more recently introduced varieties tested were susceptible as evidenced by a strong mottle or mottle-and-leaf necrosis. Tubers from inoculated hills were replanted for study of second generation symptoms. No diminution in severity of symptoms was noted in the second generation and most hills of susceptible varieties evidenced the disease. Field spread tests indicated approximately 25 per cent infection due to vine contact during the previous generation. Although the variety Ontario was rather susceptible, inoculation of about 150 individual tuber clones showed that about 25 per cent of them were highly resistant. A similar situation held with several other new potato varieties. The basis for this resistance is considered to be due to the type of the latent virus originally present in the clone, before inoculation with the virulent ringspot virus.

Isolation of virulent ringspot virus from "healthy" potatoes. LARSON, R. H., AND R. C. LADEBURG. A virulent ringspot virus was isolated readily from the ordinary latent virus universally present in older American potato varieties by means of single lesion transfers from the mottle-ringspot complex as expressed on *Nicotiana tabacum* and *N. rustica.* Isolations were made from the following varieties: Green Mountain, Russet Rural, Russet Burbank, Mohawk, Triumph, Cobbler, Houma, Red Warba, Gold Coin, and Beauty of Hebron. Physical properties included: thermal inactivation, 70° to 72° C.; dilution end point, about 1 to 1,000,000; aging in vitro, over six months. These properties checked very closely with those of virulent ringspot occurring naturally in potatoes. The ringspot virus was manifested in the form of three distinct types of local lesions on *N. rustica,* being designated as clear ringspot, necrotic ringspot, and necrotic spot. Pure cultures of the three types were also obtained by means of single lesion transfers. When inoculations were made at 16° C. to Red Warba and Pontiac with each of seven cultures of ringspot derived from the ordinary potato latent virus complex and eleven cultures of virulent ringspot isolated directly from potato, there were considerable differences between individual isolates as to virulence but the ringspot isolates derived from the latent virus complex proved fully as pathogenic, as a group, as the cultures isolated directly from potato.

Use of soluble and wettable fungicides for seed treatment. LEACH, L. D. To eliminate the objectionable features of dust application other methods of treating seed such as liquid fixation of dusts, slurry and spray treatment, have come into use. The last two methods require the use of either wettable or soluble fungicides. Numerous comparisons have shown that with uniform coverage spray applications of fungicides provide protection equal or superior to that from dust treatments. Soluble fungicides are more satisfactory for spray application because they eliminate the problem of maintaining suspension and reduce nozzle clogging. With beet seed a 0.15 per cent solution of ethyl mercury phosphate applied at 4 per cent of the seed weight was as effective against Pythium damping-off as Ceresan M (ethyl mercury p-toluene sulfon-

anilide) or Phygon (a naphthoquinone) at their usual dosages. Upon European sugar beet seed heavily infected with *Phoma betae*, Phygon XL, Arasan (a thiuramdisulfide), and Cerasan M applied as dusts provided inadequate control whereas the use of ethyl mercury phosphate as a spray (0.3 per cent solution) or a 20 minute seed dip (40 p.p.m.) resulted in over 90 per cent healthy seedlings. The maximum moisture application without drying depends upon the absorptive power of the seed surface. Four per cent is satisfactory on beet seed, but not more than 0.5 per cent should be used on Lima beans.

Resistance to anthracnose in sorghum. LeBeau, F. J. During the past two years several hundred importations of sorghum have been tested for reaction to *Colletotrichum graminicolum* leaf spot. Under conditions of natural infection and also artificial inoculation disease development ranged from 0 to 100 per cent defoliation at the ripe stage. The domestic sorghum varieties used as controls were usually 100 per cent defoliated. Importations from India were, with few exceptions, also highly susceptible. Numerous highly resistant individual collections were isolated from among the African collection, which represented the bulk of the importations. Only a flecking reaction was produced on the more resistant collections artificially inoculated in the seedling stage with a spore suspension forced between the young unexpanded leaves with a hypodermic needle and syringe. Under similar conditions severe leaf spotting and necrosis of the expanding leaves occurred in susceptible varieties. In crosses involving two resistant collections with susceptible domestic sorghum varieties the F_1 proved to be highly resistant, in this respect reacting as the resistant parent. Preliminary F_2 data indicate a 3 : 1 segregation for resistance and susceptibility.

The determination of antimycin on plant leaves. Leben, Curt. Antimycin, an antibiotic that has been studied as a protectant fungicide, may be assayed on plant leaves by means of an agar diffusion method. Plants are sprayed with an antimycin suspension, dried, and discs $\frac{1}{4}$ inch in diameter are cut at random from the leaves. Spores of the assay organism, *Colletotrichum circinans*, are seeded in acidified (pH 3.8–4.2) potato dextrose agar plates. Leaf discs are placed, sprayed side downward, on the agar surface; likewise, blotting paper discs containing different concentrations of a standard antimycin preparation are tested. Plates are incubated at 28° C. for 40–48 hours and inhibition zones around the discs measured. A curve for the standard is plotted (the diameter of the zone of inhibition is a straight line function of the log of antibiotic concentration) and the amount of antibiotic on the leaf discs calculated in terms of "leaf units" by interpolation. The acidic medium prevents the obliteration of inhibition zones by rapid growth of bacteria from leaf discs; only occasionally have fast-growing fungi from discs prevented measurements. The deposition and wash-off of antimycin have been studied by this method. Leaves of apple, elm, tomato, cowpeas, and *Lonicera tatarica* have been used.

/ *Cytoplasmic inclusions associated with wound-tumor in Rumex acetosa.* Littau, Virginia C., and L. M. Black. Cytoplasmic inclusions have been found in cells of root tumors of *Rumex acetosa* and several other species of plants susceptible to the wound-tumor virus, *Aureogenus magnivena*. They were usually located in the undifferentiated cells of the tumor, but were occasionally observed in the tumor tracheids. They resembled nucleoli with respect to size, shape and reaction to different cytological fixatives and stains. Inclusions of this type were rarely observed in non-tumorous areas of roots from which tumors developed, and very rarely in meristems of uninfected roots. When stained with Flemming's triple stain, the nucleoli and inclusions stained red with safranin, while the nuclei were violet. The inclusions and nucleoli were Feulgen-negative. When stained with Unna's pyronin-methyl green mixture adjusted to pH 7, the inclusions and nucleoli were red (pyronin) and the nuclei blue. Digestion with the enzyme ribonuclease has failed to alter the staining properties of both nucleoli and inclusions in tests carried out thus far. The evidence from staining reactions indicates that the inclusions are composed of essentially the same materials as the nucleoli.

Chemical methods for the determination of dithiocarbamates in spray residues. Mack, G. L. Two types of dithiocarbamate compounds have come into use as fungicides; the dimethyl compounds, of which the iron and zinc representatives are already well established, and the ethylene bis compounds, of which the sodium, zinc, and manganese compounds are available. The tenacity and stability of the dithiocarbamates in residues vary widely, so that performance is difficult to evaluate on the basis of disease control alone. Colorimetric methods for both iron and zinc dimethyldithiocarbamate have been developed based on their solubility in organic liquids. The ethy

bisdithiocarbamates are distinguished by their insolubility in organic solvents, instability in acids, and strong adsorption from alkaline solutions. Print methods, which require no preliminary solution or concentrations, are therefore especially indicated. They were also applicable to dimethyldithiocarbamate complexes where distribution patterns are of particular interest. The dithiocarbamate residue is transferred in a letter press from the plant surface to a hardened filter paper moistened with sodium hydroxide. A stable, highly colored, brown copper complex is precipitated in the paper on immersion in a copper sulfate solution buffered to pH 5. The relative depth of color is a rough quantitative measure of the amount of dithiocarbamate present, and forms a permanent record of the determination.

Spray adjuvants to improve the sticking and distribution pattern of zinc dimethyldithiocarbamate on tomato foliage. MACK, G. L., AND W. T. SCHROEDER. The possibility of improving the adhesion and distribution pattern of zinc dimethyldithiocarbamate (Zerlate) on tomato foliage with various adjuvants was investigated by correlating disease control data with the evaluation of the fungicide deposits as determined by a chemical leaf print method. The following commercial preparations listed as stickers or spreaders were applied with Zerlate in 5-application schedules in five randomized blocks: Orthol K at 1 pt. and at 1 qt., Orthol D at 1 qt., B 1956 at 4 oz., DuPont Spray Adhesive at 1 pt., Colloidal Z1 at 1 lb., Veg-Oil at 1 pt., and Nuflm at 1 pt. per 100 gallons of spray. Prints were made from leaves selected at random from each of three positions on the plants immediately after spraying and again after weathering. A correlation existed between leaf deposit ratings and control of Alternaria defoliation, with one exception. B 1956 deviated from the relation: its control of disease was better than would be predicted from the leaf prints. Two materials gave poorer disease control than Zerlate alone. Redistribution of the fungicide from the upper to lower leaves was indicated. Different distribution patterns also were obtained with the various adjuvants. Orthol K, Orthol D, and Veg-Oil were the outstanding materials from the standpoint of deposit ratings and control of Alternaria defoliation.

The influence of temperature on the incubation of the wound-tumor virus in the insect Agallia constricta. MARAMOROSCH, KARL. Experiments were designed to find the influence of temperature on the incubation period of the wound-tumor virus (*Aureogenus magnivena*) in the insect vector *Agallia constricta*. They were carried out in chambers with controlled temperature and light. The temperature was kept constant day and night. The day-periods were 16 hours long, night periods were 8 hours. The light source consisted of 28 fluorescent tubes per chamber, giving about 560 meter-candles. At 16° C. the minimum incubation period was 30 days. Thereafter, there were long and irregular intervals between transmissions (up to 22 days). At 26° C. the minimum incubation period was 14 days, with very regular transmission. No transmission occurred at 37° C. All checks remained healthy. Insects which completed their incubation period at 26° C. lost their transmitting ability almost completely at 35° C. and did not regain their transmitting power when returned to 26° C. When insects transmitting regularly at 26° were placed at 16° C., transmission became very poor and irregular, but returned to normal when the temperature was again raised to 26° C. A temperature of 0° C. almost stopped incubation, but upon return to 26° C. the incubation period was completed. The investigation demonstrates the importance of controlled temperatures in experiments with incubation periods in insects and shows the effect of temperature on both the incubation and transmission.

Strains of Phytophthora infestans capable of surviving high temperatures. MARTIN, W. J. Attempts to explain the unusual occurrence in recent years of late blight in the fall crop of Irish potatoes in Louisiana led to a study of the reaction of different isolates of *Phytophthora infestans* to high temperatures. Cultures of the different isolates on navy bean infusion agar were exposed at 36° C. for varying times. After exposure, the cultures were kept at 20° C. for several days after which transfers were made to determine whether they were still viable. Eight different isolates were used, including four from Louisiana, three from Minnesota, and one from Cornell. The maximum time survived by the isolates was as follows: The four Louisiana isolates, 6 days; the three Minnesota isolates, 4 days; and the Cornell isolate, less than 6 hours.

Factors influencing the sporulation of Colletotrichum lindemuthianum in culture. MATHUR, R. S. *Colletotrichum lindemuthianum* (gamma strain) does not readily sporulate on common synthetic media. A medium containing glucose, mineral salts, and neopeptone was most favorable for sporulation. When glucose was replaced with equivalent quantities of sucrose, xylose, and galactose, sporulation was equally good.

Mannose, maltose, fructose, and lactose were less favorable while sorbose, soluble starch, mannitol, and sorbitol were unfavorable. Neopeptone was by far the most favorable nitrogen source tested for sporulation. The following nitrogen sources, in order of effectiveness, allowed some sporulation: urea, glycine, arginine, asparagine, and sodium nitrate. The fungus was partially deficient for inositol and biotin. Sporulation was stimulated when small discs of vitamin starved mycelium were soaked in dilute solutions of inositol, biotin, a mixture of these vitamins with thiamin or in a water extract of spores and matrix. These results suggest that spore matrix is rich in the essential vitamins. Sporulation was best at 15° and 20° C., less abundant at 25° C., and rare at 30° C. There was no sporulation below pH 3.0. Sporulation increased suddenly at pH 3.6, was greatest between pH 5.2 and 6.5, and moderate up to pH 7.7. Light and aeration were without effect on sporulation.

Soil treatment with Arasan for the control of damping-off of certain vegetables. MCKEEN, C. D. The effect of soil treatment with Arasan (50 per cent tetramethyl thiuramdisulphide) on the incidence of damping-off on some vegetables has been studied in greenhouse flats. Under conditions of temperature and moisture favorable to the trouble, as caused by *Pythium ultimum* Trow and *Rhizoctonia solani* Kühn, soil treatment with Arasan has proved to be very effective in combating this disease in peppers, tomatoes, spinach, and cucumber. The greatest protection against damping-off in these vegetables has been obtained from a combined soil and seed treatment with the fungicide. With use of both treatments, the pre-emergence phase of the disease has been reduced to a very low level and post-emergence damping-off has rarely exceeded 5 per cent up to 16 days after emergence.

Septoria avenae on oats in Iowa. MEEHAN, FRANCES, AND H. C. MURPHY. A leaf spot and culm infection developed on oats in Iowa during 1948. Early in April, a fungus identified as *Septoria avenae* Frank was isolated from elliptical chocolate-brown lesions (0.5 × 1 cm.) on leaves of Clinton seedlings. Necrotic areas enlarged and coalesced by late June and July, but dark brown oval centers usually remained perceptible, distinguishing these leaf spots from those caused by *Helminthosporium avenae* Eidam and *Colletotrichum graminicolum* (Cos.) Wils. Perithecia of the ascogenous stage, *Leptosphaeria avenaria* Weber were occasionally present in advanced lesions. Basal leaf infection typically spread to the adjoining sheath that became grayish-brown and speckled with pycnidial aggregates. Portions of the culm beneath these areas were browned and tended to break before harvest. Cultures of the fungus were grown in yeast extract medium, and mycelial suspensions sprayed on oat seedlings. Inoculations were successful in all 36 varieties tested. Single pycnidial isolates of *Septoria avenae* f. sp. *triticea* from barley and wheat developed the *Leptosphaeria* stage in culture. Perithecia were common on culms and leaves of infected wheat. Cultures of the wheat and barley form failed to infect oats and *vice versa*. There were indications of the occurrence of *S. avenae* throughout the northern and eastern states. Cultures were isolated from Ajax and Andrew oats from Pennsylvania and New Hampshire.

Cultural and parasitic races of Cercospora arachidicola and Cercospora personata. MILLER, LAWRENCE I. Cultural races of *Cercospora arachidicola* and *C. personata*, which differ in color, rate, and type of growth were obtained by isolation from infected peanut leaves from different geographical areas in the United States in 1946 and 1947. Monosporous lines from the various cultural races have remained very stable in culture. Inoculation studies in the greenhouse and field in 1948 with different peanut varieties, indicate that several of the cultural races of both species tested differ significantly in pathogenicity. Blackish leaf lesions with sharp margins characterize those caused by one biotype of *C. arachidicola* on Spanish variety No. 146, while light brown lesions with a small yellow border characterize those produced by another biotype of the same species on Spanish variety No. 146.

An indexing method for necrotic ring spot and yellows of sour cherry. MOORE, J. DUAIN, AND G. W. KEITT. Sour cherry trees free from necrotic ring spot and yellows have been obtained by a method that combines indirect and direct indexing on sour cherry. Dorman nursery stocks were potted and forced in the greenhouse after bud-wood had been cut from each tree and placed in cold storage. In the indirect procedure each tree indexed was inoculated at bud-break with buds from Montmorency known to carry necrotic ring spot. Expression of ring spot symptoms indicated the stock was ring spot free when inoculated; no symptoms indicated the stock had ring spot when inoculated. In the direct procedure scions from storage were used to inocu-

late potted sour cherry free from necrotic ring spot and yellows. Expression of ring
spot symptoms on these trees indicated the tree being indexed was carrying necrotic
ring spot; no symptoms indicated it was ring spot free. Ring spot symptoms usually
occurred in one to three weeks at greenhouse temperatures of 20° to 24° C. Scions
from all trees shown by both procedures to be ring spot free were taken from storage
and budded to potted Mahaleb rootstocks that were breaking bud. Thus far all trees
free from necrotic ring spot have been yellows free also.

The effect of wetting agents on brown rot control. MORGAN, OMAR D., AND DWIGHT
POWELL. Copper 8-quinolinolate was selected from several chemicals by laboratory
tests especially designed for this experiment as the best fungicide to use in field tests
against Sclerotinia fructicola on peach. Three proprietary wetting agents, each at
300 p.p.m. and 20 per cent alcohol were used with this compound. After spraying the
peaches, which were nearing the firm ripe stage of maturity, all trees were inoculated
with a suspension of Sclerotinia fructicola spores. This inoculation was done after
darkness with a power sprayer at 300 lb. pressure. Degrees of infection were recorded
from field counts and from peaches brought into the laboratory. "Triton B 1956"
was the most effective wetting agent with copper 8-quinolinolate and allowed 12 per
cent brown rot. Copper 8-quinolinolate when used alone resulted in 28 per cent infec-
tion. "Santomerse S," "Sterox CD," and 20 per cent alcohol resulted in 19, 16, and
20 per cent infection, respectively. The check plot which received no chemical treatment
had 38 per cent brown rot infection.

Leaf rust resistance within certain species and hybrids of Populus. NAGEL, C. M.
Cotton-woods, which are an important tree species in shelterbelt plantings of the Plains
States, frequently suffer a high mortality following planting. Although the causes of
mortality are perhaps several, it appears that leaf rust, Melampsora medusae Thüm.
is an important factor in survival. Leaf rust frequently causes complete defoliation
anytime after midseason on Populus deltoides Marsh., the principle species grown in
shelterbelts throughout the Plains States. The effects of extensive defoliation on car-
bohydrate reserves and its association with ultimate winter killing or hardiness is pres-
ently incompletely known. During the past several years approximately 250 selections
consisting of clonal lines of Populus deltoides, P. sargentii, and hybrids involving nu-
merous species of Populus have been obtained and tested for their reaction to cotton-
wood leaf rust. Certain of these clonal lines possess high resistance to leaf rust. The
range in defoliation was from 97.7 to 6.5 per cent, indicating a wide range in their
tolerance to this pathogen. Certain of these hybrids appeared to have immunity to
the currently prevalent leaf rust fungus under field conditions.

Comparison of dust fungicides and formaldehyde in the control of onion smut.
NELSON, RAY. Experiments from 1943 to 1947 comparing dust fungicides with formal-
dehyde revealed the superiority of the liquid treatments in the control of onion smut
in heavily infested soil. Denser stands and satisfactory control of smut were obtained
in moderately infested soil with Arasan at the rate of 1 lb. to 4 of seed mixed in the
drill. Seeds pelleted with Arasan and other fungicides at rates of 1-4, 1-2, and 1-1
gave unsatisfactory control and caused injury manifest in thin stands. Other dust
fungicides have been tested in pellets and in the drill mixed with the seed. With the
exception of Dithane Z-78 (disodium ethylene bisdithiocarbamate), none has equalled
Arasan (tetramethyl thiuram disulfide) in effectiveness and safety. Bridging in the
drill limits the maximum application of Arasan mixed with the seed at 1 to 4. A drill
was designed to dispense any desired amount of dust simultaneously with the seed. In
1948 with soil conditions exceptionally favorable for smut, Arasan and Dithane Z-78,
diluted 1 to 3 with talc and dispensed at the rate of 2 lb. to 1 lb. of seed, gave su-
perior control of smut and damping-off and increased stands 63 and 73 per cent, re-
spectively, over those from the undiluted dusts applied 1 to 4.

Life history of Stemphylium causing red leaf spot of gladiolus. NELSON, RAY,
AND MARIE MOOAR. Stemphylium sp. has been isolated from gladiolus leaves from
Michigan, California, Florida, New York, and New Jersey. One New York isolate of
September 16, 1946, on prune agar formed much larger stromata than other cultures
and was subjected to varying environmental conditions, including freezing and drying.
April 21, 1948, 19 months after seeding with Stemphylium sp. the plate contained
Pleospora perithecia with mature asci. Single-spore cultures were obtained from asco-
spores expelled to the cover of the Petri plate. Typical Stemphylium red leaf spot re-
sulted from infection of gladiolus leaves with these cultures. Re-isolates from the
lesions formed much larger stromata than previous cultures. The perithecia are glo-

bose, irregularly clustered, and densely setose. The outer cells are thickened and black. Perithecia are 396–555 × 460–904, average 495 × 725 microns. The asci are cylindrical, thin-walled with a thick, gelatinous inner wall. Ascospores are 11–30 × 29–70, average 17 × 42 microns, and vary from light, transparent yellow-brown to dark red-brown. The number of cross and vertical ascospore walls varies greatly within one perithecium. Species determination has been deferred.

Potato virus disease research in Mexico. NIEDERHAUSER, JOHN S.

Inheritance of sex and pathogenicity in Ceratostomella fimbriata from sweet potatoes. OLSON, E. O. *Ceratostomella fimbriata* has been considered a homothallic species. Among ascospore isolates from self-fertile (perithecial) strains a number of self-sterile (non-perithecial) strains were obtained. Two of these were of particular interest as they formed perithecia where they grew together. One, named self-sterile 1, produced sclerotium-like bodies, functioned as a female strain, and was pathogenic to sweet potatoes. The other, named self-sterile 5, produced no sclerotium-like bodies, functioned as a male strain, and was not pathogenic to sweet potatoes. Ascospores from the perithecia formed by these two strains gave four strains in a 1:1:1:1 ratio. They were the two parental strains, the self-fertile strain from which the two self-sterile strains were originally obtained, and another self-sterile strain which was neuter. Pathogenicity and non-pathogenicity were inherited in a 1:1 ratio, the self-sterile 1 and self-fertile strains being pathogenic and the self-sterile 5 and new strain being non-pathogenic.

Relationship of Ceratostomella fimbriata from the Hevea rubber tree and sweet potato. OLSON, E. O., AND W. J. MARTIN. Self-fertile (perithecial) cultures of *Ceratostomella fimbriata* isolated from *Hevea brasiliensis* in Mexico and *Ipomoea batatas* from the United States were very similar morphologically but differed in pathogenicity. The isolates from *Hevea* were not pathogenic to sweet potato and the isolates from sweet potato were not pathogenic to *Hevea*. The two were alike in that they both gave rise to self-fertile and self-sterile strains among single ascospore cultures isolated from them. When a week-old culture of a self-sterile strain from sweet potato was spermatized with spores from a self-fertile culture from *Hevea*, perithecia formed within three days. Very poor germination occurred in the ascospores from these perithecia since only 2 of 300 ascospores isolated produced cultures. The two cultures were self-fertile. One was pathogenic to both *Hevea* and sweet potato while the other was pathogenic only to *Hevea*. Ascospores isolated from the two cultures gave self-fertile and self-sterile strains with the same pathogenicity as the parent. The evidence indicates that hybridization occurred between the sweet potato and *Hevea* forms of *C. fimbriata*.

Induced variations in two species of Botrytis causing neck rot of onion. OWEN, J. H. Treatment of spores from *Botrytis allii* with methyl-bis (B-chloroethyl) amine produced several mycelial variations similar to *Botrytis byssoidea*. These variations remained mycelial for 3 to 4 transfers before sporulation reoccurred. Similar treatments of spores from *B. byssoidea* resulted in mycelial variations of different growth rate. One of these variants when inoculated into onion bulbs developed spores much smaller than normal and nearer to the spore size of *B. allii*. A large variety of media containing onion extracts and many vegetable decoctions failed to induce sporulation in *B. byssoidea*, whereas *B. allii* sporulated readily on all media prepared. One isolate of *B. byssoidea* produced sclerotia on potato-dextrose agar at 4°–12° C. in 30 days; addition of 0.5 per cent uranium nitrate induced sclerotium formation at higher temperatures. The same treatment with uranium nitrate caused a reduction in the growth rate of *B. allii*, but had no effect on sclerotium formation.

Some histological relationships between Helminthosporium victoriae and a susceptible and a resistant oat. PADDOCK, W. C. The reactions of Vicland, a susceptible variety, and Clinton, a resistant variety, of oats, when inoculated with spores of *Helminthosporium victoriae*, were investigated by the use of cotton-blue stained, free-hand sections of seedling leaves which had been dusted with spores and placed in a moist chamber. Spores germinated by the formation of one or more terminal germ-tubes. One to three appressoria were usually formed at the terminals of the germ tubes. On Vicland, the primary hypha may pass intracellularly through the epidermis and thereafter, until the later stages, is mostly intercellular. Occasionally the primary hypha is bulbous and thick and is limited for some time to the epidermal cells. In cells adjacent to the hyphae, the chloroplasts are nearly always disintegrated. On Clinton oats the primary hypha is limited to the epidermis and is frequently no larger than the germ

pressorium. When it is larger, it has a diffuse granulated appearance with poorly differentiated walls. Disintegration of the primary hypha begins some time after penetration so that 80 hours after infection it has a clear amorphous appearance much like cytoplasm of the suscept cell. No disorganized chloroplasts were found in Clinton.

The effects of Fermate on the yield of McIntosh apples. PALMITER, D. H. The continuous use of Fermate (70 per cent ferric dimethyldithiocarbamate) on McIntosh apple trees which received no nitrogen fertilizer over a period of six years increased the yield of fruit 57 per cent over that of corresponding trees that were sprayed with wettable sulfur. Annual soil applications of two or more pounds of N per tree increased the yield of sulphur sprayed trees over that of the unfertilized Fermate plots during the first two years of the test but in the last three years the Fermate plots have outyielded even the best nitrogen fertilized plots. In 1947 and 1948 the best sulfur sprayed and nitrogen fertilized plots averaged 9 boxes of fruit per tree per year compared with 17 boxes of fruit per tree per year from the Fermate plots. Several factors appear responsible for this increased yield where Fermate is used as the fungicide in place of sulfur. Fermate contains some nitrogen and in a spray season a tree may receive as much as $\frac{1}{4}$ pound of N. However, other factors such as reduced scab and spray injury to the foliage may be just as important. In 1948 most of the trees in the experiment had close to 100 per cent bloom, but blossoms and fruit counts indicated more than 50 per cent increase in fruit set on the Fermate sprayed trees.

Physiologic specialization and variation in Helminthosporium teres. PON, DICK S. *Helminthosporium teres* is composed of many cultural races that may be distinguished by the following characteristics: nature and amount of mycelial growth, color, zonation, rate of growth, size and shape of spores. Mutation in culture is not uncommon in certain races. The pathogenicity of certain races differs sharply on varieties of barley. The optimum temperature for growth of the organism in culture is 25° C., for infection about 20° C.

Peach brown rot: pre-harvest sprays and control in the orchard and in the package. POULOS, P. L. AND J. W. HEUBERGER. An experiment on the Halehaven variety was conducted to determine the relative effect of various fungicides on rot in the orchard and subsequent rotting in the package. Incidence of rot was estimated as 3 per cent when the first pre-harvest spray was applied. Four sprays were applied (July 10, 16, 22, 30); four rot counts were made (July 24, 28, 30, Aug. 3); and three harvests were made (July 28, July 31, Aug. 3). At each harvest, the fruit was brushed, graded, packed in open-staved bushel baskets, stored at room temperature (approximately 75° F.) for 3 days and rot counts made. Percentage rot in the orchard for the non-treated, Liquid Lime Sulfur (2 qt.-100), Liquid Lime Sulfur + B-1956 (2 qt.-1 oz.-100), Sulforon (a wettable sulfur) + B-1956 (4- 1 oz.-100), and Bioquin 1 + B-1956 ($\frac{1}{2}$-1 oz.-100) was 38, 34, 25, 23, and 14, respectively, while percentage rot in the basket was (average for the 3 harvest dates) 72, 54, 56, 45, and 38, respectively. Liquid lime Sulfur and Bioquin 1 were phytotoxic to the foliage. Bioquin 1 (copper-8-hydroxy-quinolinolate) imparted a "rusty" color to the fruit. An experiment on the variety Elberta yielded similar results. The dithiocarbamates, Parzate, Dithane Z-78, and Zerlate, were relatively ineffective for control in the orchard and in the package. Various soluble dithiocarbamate fungicides failed to control rot in the orchard and were highly phytotoxic to foliage. A combination of Liquid Lime Sulfur + Sulforon (1 qt. 2-100) appeared promising.

Effect of air temperature on virus concentration and leaf morphology of mosaic-infected horseradish. POUND, GLENN S. A strain of turnip virus 1 inciting horseradish mosaic was found to occur in much greater concentration in horseradish grown at 16° C than at 28° C. This was also true for old leaves of rape plants infected with the horseradish virus, but in young leaves the concentration was higher at 28° C. When horseradish plants growing at different temperatures and showing a gradient of virus concentration were reversed in position, i.e., plants moved from 16° to 28° and vice versa, a corresponding reversal in virus concentration occurred. At temperatures where virus concentration was low, symptoms were very mild or masked; where virus concentration was high they were severe and persistent. Temperature also produced a marked effect on leaf morphology. At 28°, the prevailing leaf type was broadly laminate; at 16° leaves were often reduced to extreme fern-leaf structures. At intermediate temperatures, intermediate leaf types were produced. A reversal in temperature resulted in a corresponding reversal in leaf morphology. There was no correlation between leaf type and virus concentration. Efforts to free horseradish roots and plants by treatments in hot water and hot air, respectively, were unsuccessful.

Watermelon mosaic in Wisconsin. POUND, GLENN S. For the past 3 years, watermelon and muskmelon plantings in a south central Wisconsin area have been affected with a virus disease. Ten per cent of watermelon plants were infected in some fields. Symptoms on watermelon consist of severe stunting, bud proliferation, irregular black necrotic lesions, and occasional mottling. On muskmelon, symptoms are very similar to those produced by cucumber mosaic viruses. On all varieties of pea tested, marked systemic mottling was followed by death. On bean, the virus produced characteristic local necrotic lesions and systemic top necrosis. Symptoms on Havana tobacco occurred as etch-like rings and ringspots or as an oak leaf pattern on a conspicuously yellowed leaf. The virus was widely infectious to hosts tested. In physical property studies inactivation occurred at: dilution, 1–100,000; ageing in vitro, 5 days; thermal inactivation, 70° C. Cross immunity tests indicated no relationship with either cucumber virus 1 or tobacco virus 1. In certain symptoms, properties, and host range, the virus appears similar to the tobacco ringspot virus.

Fruit tree disease control with a new type of spray-duster and mist sprayer. PRATT, ROBERT M., L. M. MASSEY, AND K. G. PARKER. A sprayer for fruit trees, developed at Cornell University, applies either wetted dusts or mist spray formulations. The spray, or spray-dust, is carried by an air stream delivered from a 3 inch slot 8 feet long set at an angle of 35° from the horizontal. The air delivery of the machine now under test is approximately 20,000 c.f.m. at 105 m.p.h. Means are provided to adjust the angle of the air streams to compensate for wind interference. There is a saving of water of approximately seven-eighths of the amount used in conventional dilute spraying. Control of apple scab, cherry leaf-spot, and peach leaf curl has been equal or superior to that obtained by conventional methods. Sulfur, lime sulfur, low soluble copper compounds, ferric dimethyl dithiocarbamate, 2-heptadecyl glioxalidine, dinitro ortho cresol, and insecticides and adjuvants have been applied. Experiments on the control of fruit insects have been made in cooperation with entomologists. Both the spray-dust and mist spray methods are effective, but the latter is more economical of materials and is mechanically simpler. Disease control and chemical deposit data indicate that adequate coverage is obtained with this machine, which offers advantages over conventional sprayers in ease, speed, and economy of operation.

Fungicidal activity of dinitrocaprylphenyl crotonate. RICH, SAUL, AND JAMES G. HORSFALL. 2,4-Dinitro-6-caprylphenyl crotonate (code name Cr 1639) is an effective acaricide which gave good control of potato late blight in the 1945 insecticide field trials of Dr. F. B. Maughan. Used at 2 lb. per 100 gallons, during the 1946, 1947, and 1948 seasons Cr 1639 gave fair control of bean anthracnose, of *Septoria* and *Cereospora* blights of celery, and good control of early apple scab. It produced some leaf scorch on all test plants except celery. Laboratory and greenhouse tests showed that the tenacity and phytotoxicity of Cr 1639 are due to the dinitrocaprylphenyl portion of the molecule, while the fungitoxicity is due to the crotonic acid portion. In the laboratory, a deposit of Cr 1639 on glass is fungitoxic before drying but not after drying. Upon drying, Cr 1639 hydrolyzes and loses crotonic acid by volatilization. For this reason the usual laboratory screening techniques would miss this compound. Toxicity was reasonably maintained by drying the deposit on a strip of leaf epidermis. In the field, Cr 1639 probably acts mainly as a therapeutant, and as a protectant only so long as the leaf cuticle can retain the crotonic acid. Possibly crotonic acid can be used as a therapeutant in spray programs for apple scab and other diseases.

The western "X" virus a cause of "little cherry" in Utah. RICHARDS, B. L., L. M. HUTCHINS, and E. L. REEVES. Transmission studies during the past two years have established the fact that "little cherry", as it is recognized in Utah, is an expression of the Western "X" virus in the sour and sweet cherries on Mazzard root stock. When buds and fruit spurs from sweet and sour cherry trees with "little cherry" symptoms are grafted into peach, characteristic leaf symptoms of Western "X" disease of the peach result; when grafted into sweet and sour cherries on Mahaleb root stocks, typical wilt and decline symptoms are produced; and when grafted into chokecherry (*Prunus virginiana* and *P. demissa*), Western "X", red leaf, and "little cherry" fruit symptoms develop. These results with the "little cherry" inoculum duplicate in every detail results obtained in previous years with Western "X" virus inoculum from both the peach and the chokecherry. The transmissibility and general nature of the "little cherry" in Utah is further shown by the fact that buds and fruit spurs from diseased sweet or sour cherry trees grafted into sour cherry on Mazzard roots induced "little cherry" fruits, rosetted leaves, and proliferation and persistence of stipules.

Ozone as a stimulant for fungus sporulation. RICHARDS, M. C. Ozone is generally considered to be a fungicide and is used in meat storage plants to retard the development of fungi. It has been used experimentally in apple storages for similar reasons. Certain fungi which produced few or no spores in culture when exposed to ozone produced spores abundantly. Sporulation was increased with *Alternaria solani* and *Alternaria oleraceae* with no impairment of germination. A third species of *Alternaria*, which did not produce spores normally in culture, produced numerous spores when growing cultures were exposed to ozone. Ninety-six per cent of the spores germinated. The squash black rot pathogen, *Mycosphaerella citrullina*, produces few or no pycnidia nor spores on potato-dextrose agar. When cultures are exposed to ozone, masses of spores are produced, none of which will germinate. Ozone will stimulate fungi to sporulate. With certain fungi, however, ozone appears to be toxic to the spores.

The vacuum duster for applying fungicides and inoculum to plants. RICHARDS, M. C. AND DOUGLAS MURPHY. The application of dusts to bean plants to control bean rust appears to be a useful technique in screening fungicides. The vacuum duster which is in general use in the screening of insecticides has been found to give a uniform distribution of rust inoculum and fungicide to both the upper and lower surfaces of bean leaves. The cotyledon leaves of the variety Pinto were used in the tests. Fifty milligrams of the diluent containing 0.5-per cent uredospores were best for making the inoculations. With some of the more active mixtures 50 mg. of a one-per cent concentration gave complete control. The poorer mixtures required 50 mg. of a 4-per cent concentration to give equal control. The low percentage of the active ingredient in the dusts necessary to give complete control illustrates the importance of good coverage. By observing the number and distribution of the rust lesions it was possible to determine differences in the nature of the diluents with respect to ease of mixing and uniformity of the mixtures.

Selecting white pine for resistance to blister rust. RIKER, A. J., T. F. KOUBA, AND W. H. BRENER. In 1938 and 1939 selections were made in four Wisconsin areas of 163 cone-bearing white pine trees. They had been exposed about 15 years to blister rust among Ribes having from 10,000 to 65,000 feet of live stem per acre. Over 1,000 grafts from them on 2–2 pine seedlings and 10,000 seedlings were planted. They grew vigorously in a rust nursery also planted with many Ribes bushes. Furthermore, half the trees were inoculated individually, placed under muslin cages, and kept moist for over 60 hours. After such severe inoculation over 99 per cent of the control seedlings were killed by blister rust. Most of the parent trees (as grafts) had more or less resistance; about one-fourth possessed high resistance. Such resistance occurred in one or more of these ways: (1) infection negative or reduced in amount, (2) needle lesions reduced to small spots or flecks, (3) stem lesions corked out (the rust fungus apparently died), (4) lateral stem lesions failed to reach the main stem before the twig died, and (5) cankers failed to form aecia. Seedlings from these wind-pollinated selected trees, however, had little more resistance than commercial seedlings.

Oat parentage and procedures for combining resistance to crown rust, including race 45, and Helminthosporium blight. ROSEN, H. R. Since all known Victoria derivatives that are highly resistant to race 45 of *Puccinia coronata* are susceptible to Helminthosporium blight, and since Bond derivatives, with some notable exceptions, are susceptible to race 45, while resistant to blight, the problem of combining resistance to the two has been met: (1) By incorporating new genes into Victoria or Bond derivatives as by crossing with Mutica Ukraina or other varieties which possess resistance to one or both diseases; (2) by utilizing those Victoria derivatives which, while exhibiting an intermediate but apparently genetically fixed reaction to race 45, are largely resistant to blight as in selections from the crosses Boone × (Harry Culberson-Fulghum w.t.) and (Fulghum-Victoria) × (Bond-Iogold) ; (3) by using Bond derivatives that show combined resistance as occasionally found when a Fulghum type is used as one of the parents; and (4) by utilizing mixed populations of any one cross and not breeding for uniformity. As an example of the last, selections have been obtained from the variety Traveler (Victoria × Custis) in which approximately 70 per cent of the plants are resistant to race 45 and susceptible to blight, while the reverse is true for 30 per cent of the plants. Other selections present different proportions.

Factors affecting lesion formation on Physalis floridana inoculated with potato virus Y. ROSS, A. FRANK. A study was made of the factors influencing necrotic lesion formation on plants of *Physalis floridana* Rydb. inoculated with potato virus Y (*Marmor upsilon* H.). Diluted juice of diseased plants of *Nicotiana glutinosa* L. was used in all tests. Lesion formation was favored by partial shading and by tem-

peratures between 17° and 21° C. The use of carborundum increased lesion-count about eight-fold. Continued use of inoculating pads without renewal of inoculum resulted in a rapid progressive reduction in number of lesions. About six strokes per half-leaf was found to be optimal. There was a progressive reduction in lesion-count as the time between inoculation and rinsing of leaves was increased. Several buffers and water were compared as diluents. Although borate buffers caused severe injury to leaves unless removed by rinsing within a minute following inoculation, the use of 0.1 M borate pH 8 gave more lesions than did that of any other diluent. This was followed in order by 0.1 M glycylglycine pH 7 and 0.005 M phosphate pH 7. Dilution curves showed an increase in lesion-count up to a dilution of about 1–10. This was followed by a decrease approximately proportional to the decrease in inoculum.

Studies of "Mist Blower" fungicidal concentrates for row crops. ROWELL, J. B. AND F. L. HOWARD. The application of fungicidal concentrates to row crops at rates of 3–5 gallons per acre by low-volume applicators has been investigated to evaluate this principle for disease control. Field tests in 1947 of oil-soluble fungicides applied with a modified Potts-Spencer "Mist Blower" demonstrated the necessity of fine particle size and a wide distribution pattern for coverage and avoidance of injury. Laboratory studies demonstrated that 2–4 times LD 90 of oil-dissolved fungicides could be safely applied to excised tomato leaflets as 4 μ droplets. A Potts-Spencer skid-mounted airblast machine attached to a Case VI tractor was employed in the current season's tests. An adjustable 4-row boom was equipped with 4 fish-tail air nozzles in front of which were mounted 4 Fitzhenry-Guptill VS-67 liquid nozzles that sprayed the concentrate into the air stream. The droplets varied considerably in size. Large droplets of the oil-fungicide solutions severely injured the foliage. Injury was eliminated by emulsifying the fungicide-oil solution in water. Two organic copper salts, Procop 110 and Puratized 111–5, satisfactorily controlled late blight of potatoes as compared to the checks; yields were 275, 266, and 227 bushels per acre respectively.

Comparison of volatile soil fungicides. SCHMITT, C. G. Of over 600 soil fungicides tested in soil in pots at 80° F., twelve of the more effective volatile fumigants were compared to determine approximate minimal lethal dosages in soil for vegetative mycelium of *Fusarium dianthi*, *F. oxysporum* var. *lycopersici*, *Sclerotium rolfsii*, *Scl. delphinii*, *Phoma terrestris*, *Verticillium albo atrum*, *Pythium de Baryanum*, and *Rhizoctonia solani*. Chloropicrin (Larvacide), the most effective of the group, killed all of the test fungi at 7.6 gallons per acre when sealed in and at 42.6 gal. per acre under a water seal. Minimal lethal dosages for other effective fumigants were 61 gal. for allyl bromide and 76 gal. for allyl and ethyl isothiocyanate; 380 gal. for formalin, Iscobrome 1 (15 per cent MeBr in xylene) and D-D (mixture of 1,3-dichloropropene 1 and dichloropropane), and 456 gal. for allyl chloride and methyl bromoacetate. The above dosages may not be adequate for kill if large sclerotia are present.

Relation of the European corn borer to stalk rots of corn. SCHNEIDER, C. L. AND J. J. CHRISTENSEN. Presence of the European corn borer (*Pyrausta nubilalis* Hübner) in Minnesota complicates the problem of developing varieties of corn resistant to stalk rots. In 1947 considerable stalk rot accompanied corn borer injury in inbred lines and varieties of corn that had been comparatively free from stalk rots before the borer spread into the State. Rot developed in 80 per cent of the internodes injured by the borer, while noninfested internodes in the same stalks were free from rot. The borer provides ways of entry for fungi; movements of larvae distribute fungi inside the plant; the frass within larval tunnels furnishes an excellent medium for rapid growth of fungi; and larval injuries weaken the stalk tissues and increase the likelihood of fungal attack. Larvae usually are infested both internally and externally with microorganisms, including many of those that cause stalk rot.

The control of blossom-end rot of tomato with emulsified hydrocarbon sprays. SCHROEDER, W. T. A greenhouse experiment conducted during the spring of 1948 indicated that an emulsified hydrocarbon preparation (Dowax 222) might be of practical value for the control of blossom-end rot of tomato in the field. Accordingly, 40-plant plots consisting of no treatment, Dowax 222 at 1.0, and Dowax 222 at 2.0 gallons per 100 of water were arranged in random order in each of four blocks. Sprays were applied at the rate of 200 gal. per acre on July 8 and 16. Blossom-end rot counts made during the harvest season showed 55 per cent control for the wax emulsion at the lower concentration and 70 per cent at the higher. Yields of 14.3, 16.0, and 17.8 tons per acre of number 1 equivalent fruit from plots with no treatment, Dowax 222 at 1.0, and Dowax 222 at 2.0 gal., respectively, were correlated significantly with the control of

blossom-end rot. No significant differences among treatments were obtained with respect to number of harvested fruit per plant, size of fruit, or percentage of culls exclusive of that occasioned by blossom-end rot, indicating that the increase in yield was due to the control of the disease.

Transmission of purple-top of potatoes. SELF, R. L. AND H. M. DARLING. During the summer of 1948 purple-top was transmitted by bud grafts from naturally-infected Katahdin, Chippewa, and Sebago potato varieties to 33 of 67 Sebago plants in a field having only a trace of the disease. Early symptoms of the disease appeared in 35 days and were followed in 10 days by severe plant wilt and production of flabby tubers. Plants having purple-top in general field plantings were placed in large pots in insect-proof cages. Approach grafts to these plants resulted in transmission of purple-top to 11 of 55 healthy Katahdin and Sebago plants and symptoms of aster yellows were obtained on 6 of 123 *Nicotiana rustica* approach grafts. In the greenhouse, aster yellows was transmitted by dodder grafts to 3 *N. rustica* plants from 1 purple-top Sebago. During 1947 and 1948 viruliferous leafhoppers (*Macrosteles divisus*) occasionally transmitted purple-top to potatoes; only 5 definite and 10 questionable plants of 238 inoculated showed symptoms. Transmission of purple-top resulted from insect inoculation of aster yellows to the *N. rustica* half in one of 13 *N. rustica*-Sebago approach grafts with the *N. rustica* showing aster yellows symptoms before the appearance of purple-top on the potato.

The effect of low temperature exposure on the development of peach brown rot in common storage. SHARVELLE, ERIC G. AND C. L. BURKHOLDER. During the past three seasons brown rot has caused serious losses to the Indiana commercial peach crop in storage. Common fungicides applied in the orchard have not adequately prevented these storage losses. Comparable samples of peaches were harvested in duplicate from several orchards in 1948. One sample was placed immediately in common storage at room temperature, and the other sample was exposed to a 24-hour cold storage treatment at 40° F. before being placed at room temperature. The incidence of brown rot development was recorded daily over a 6-day period following harvest. In samples obtained from commercially sprayed orchards, cold storage treatment greatly reduced the subsequent development of brown rot in common storage. Brown rot losses were reduced from 41.8 per cent four days after harvest to 0.0 per cent in one commercial sample. Similar effects of cold storage were obtained with fruit sprayed with Phygon (2,3-dichloro-1,4-naphthoquinone). This evidence is opposed to the view commonly held by growers and fruit handlers that brown rot development is accelerated when fruit is removed from cold storage to room temperatures.

Effect of copper and certain dithiocarbamate fungicides on the control of Verticillium spot and bubble of mushrooms. SINDEN, J. W. AND JOHN B. YODER. Bordeaux mixture, Copper A (copper oxychloride sulfate), Fermate (ferric dimethyl dithiocarbamate), Zerlate (zinc dimethyl dithiocarbamate), Parzate (zinc ethylene bisdithio-carbamate), and Dithane were sprayed on mushroom beds in varying concentrations and at different stages in the development of the mushroom crop. Bordeaux mixture and Copper A injured mushrooms on which they were sprayed, and neither was effective in control of either Verticillium spot or bubble. Fermate, Zerlate, and Parzate caused no injury to mushrooms on the beds in concentration of one lb. per 100 gallons of water. These materials reduced the incidence of bubble by 50 per cent and of spot by much more than this. Another notable effect was the suppression of surface growth of mycelium on the bed without reducing yields. This is desirable under conditions prevailing in a mine. Parzate proved the most effective and is being used commercially by making three applications of 0.5 lb. per 100 gal. sprayed in amounts of one qt. per 10 sq. ft. of bed. The first application is made soon after casing soil is applied, the second between first and second breaks of mushrooms, the final one between the second and third breaks.

Some specific characters of Erwinia atroseptica and Erwinia carotovora. SMITH, WILSON L. JR. Fifty-eight isolates of the *Erwinia* soft rot group were used in these studies. The majority were isolated in the last few years from various decaying vegetables and from blackleg of potatoes. The group was divided into two species *E. atroseptica* (van Hall) and *E. carotovora* (Jones) Holland. The two species reacted differently in culture, mainly on their utilization of certain carbon compounds in synthetic media. *E. carotovora* utilizes ethyl alcohol (5 per cent), dulcitol (1 per cent) and sodium salts of uric, hippuric, and malonic acid at 0.15 per cent concentration. *E. atroseptica* isolates do not utilize these carbon compounds. Both species grew well on

a medium containing 1 per cent maltose, but *E. atroseptica* produces stronger acid in this medium than *E. carotovora*. All isolates rot potato slices. Only isolates of *E. atroseptica* produce blackleg of potato plants. These cultures were also transmitted on an artificially infested knife used in cutting healthy seed pieces. When such seed were planted, some rotted, resulting in missing hills, and some produced plants affected with blackleg. *E. carotovora* isolates were not transmitted in this manner. It is concluded that the blackleg organism, *E. atroseptica* should be considered a distinct species from the soft rot organism, *E. carotovora*.

Selenophoma spot, a new wheat disease for North America. SPRAGUE, RODERICK. *Selenophoma donacis* var. *stomaticola* (Bauerml.) Sprague and A. G. Johnson appeared suddenly in moderately severe form on wheat, an unreported host, in extensive areas in the Palouse country of Washington and Idaho. The fungus caused pinkish buff, irregularly elliptical leaf spots in March, 1948 and later appeared in more severe form in June as an ashy circular leaf spot with a darker, narrow margin. Artificial inoculation resulted in a few sterile spots on Federation wheat and Kentucky bluegrass but none on smooth brome, oats, barley, or Canada bluegrass. The fungus is tentatively called race 12, of variety *stomaticola*. Varietal resistance in wheat to this race, varied from the highly susceptible Rex and Orfed, to the highly resistant Kharkof, Comanche, and Hymar × Elgin 3 (F, Composite).

Recent changes in prevalence of physiologic races of Puccinia graminis tritici in South Central Mexico. STAKMAN, E. C., J. G. HARRAR, W. Q. LOEGERING, AND N. E. BORLAUG. It appears that races 17 and 56 of *Puccinia graminis tritici*, among the most prevalent races in the United States and Northern Mexico since 1939 and 1934, respectively, have now become firmly and widely established in South-Central Mexico also, where they were found only sporadically or not at all prior to 1944. Together they comprised 5, 13, 15, 28, and 34 per cent of the total isolates obtained annually in South Central Mexico from 1944 to 1948, inclusive. Prior to 1945 races other than 17 and 56, almost exclusively races 19, 38, and 59, made up 93 per cent or more of the total isolates each year. From 1945 to 1948, however, the percentages were 87, 85, 72, and 66. In 1948 races 17 and 56 comprised 25 and 10 per cent, respectively, of all isolates; and both were widely distributed, although apparently most prevalent in and near the State of Guanajuato, where stem rust was destructively epidemic. There are important implications for the program of developing stem rust resistant varieties, as certain varieties hitherto resistant in South-Central Mexico will henceforth be susceptible if races 17 and 56 persist.

Electron microscope evidence on the mechanism of multiplication of tomato bushy-stunt virus. STEERE, RUSSELL L. AND ROBLEY C. WILLIAMS. Preparations of partially purified virus from *Datura meteloides* plants in the early stages of infection with tomato bushy-stunt are found to possess particles which suggest the mechanism of multiplication of this virus. These preparations are made using a relatively mild treatment which requires only 25 minutes from picking of the infected leaves to drying of the suspension on electron microscope screens. The procedure involves dilution, mild heating, adsorption of normal plant material onto Cellite, and centrifugation at 3,000 r.p.m. Preparations from month-old infections contain spherical particles of uniform size. Electron micrographs of preparations from young infections show occasional particles which vary from this normal uniform size, and range from those which are but slightly larger and have a noticeable rift down the middle to those which are distinctly double consisting of two full-sized particles.

Studies on penetration and infection of sugarcane stalks by Physalospora tucumanensis. STEIB, R. J. Previous results have indicated that the scales on the buds of sugarcane stalks become infected with the red rot organism (*Physalospora tucumanensis*) in a latent form. The organism has been cultured from buds which were surface sterilized in a bichloride of mercury solution for as long as 24 hours. Buds with infected scales develop red rot under favorable conditions. Penetration of the epidermis of bud scales by infection threads was observed 33 hr. after inoculation with conidial suspensions. A cap or overgrowth around the infection thread was found in both a resistant and a susceptible variety. Mycelium was observed in cells below the epidermis only where the epidermal cells were thin-walled. In the resistant variety studied, the cap was seen in epidermal and sub-epidermal cells. A gummy dark red material often filled the intercellular spaces below the point of infection. This material was present but in less amount than in the susceptible variety studied. The leaf sheaths of the young shoots of a susceptible variety became infected from these bud scales. Such infected buds often failed to germinate.

Malachite green suppresses symptom expression of tobacco mosaic virus in tomato. STODDARD, E. M. AND A. E. DIMOND. Bonny Best tomatoes grown in sand and fertilized with modified Hoagland's solution were watered with a solution of malachite green in water at a strength of 1: 4000. Applications were made on 10 successive days and on the day following last application, plants were inoculated with tobacco mosaic virus. One month after inoculation, only two of five plants showed slight symptoms of tobacco mosaic virus whereas 5 of 5 check plants had pronounced symptoms. Severity indexes of symptoms (maximum index: 100) were 95 for the check plants and 15 for plants watered with malachite green. Similarly striking differences have been obtained in repeated tests. These results confirm on growing plants those reported by Takahashi (Science 107: 226, 1948) in which tobacco mosaic virus was shown not to multiply in tobacco leaves in floating leaf culture when injected with malachite green.

Utilization of the Baermann method as a means of assay of root infection by meadow nematodes, Pratylenchus spp. TARJAN, A. C. In studies of the effect of selenium on meadow nematode infections in boxwood roots, it was found that an improved method for evaluating the effectiveness of therapeutical treatments was needed. A review of the literature yielded only the tedious method of dissecting out and counting the nematodes contained in the root tissues. Tests were therefore conducted to evaluate the usefulness of the Baermann apparatus in obtaining quantitative determinations of nematode populations in infected boxwood roots. Standardized weights of infected roots were immersed in Baermann funnels, and meadow nematodes were observed evacuating the roots for as long as nine weeks. Only 19.4 ± 1.1 per cent of the total number of nematodes evacuated the roots by the first day. Most satisfactory results were obtained by drawing off aliquots three times weekly for a period of three weeks when it was found that 95.4 ± 3.5 per cent of the total number of meadow nematodes had evacuated the roots. It was concluded that a three-week immersion of the roots in the Baermann funnels would furnish a usable assay of a therapeutic treatment in the case of the meadow nematode infecting boxwood roots.

A storage scald of apples caused by midsummer oil sprays. TAYLOR, C. F. The application of spray oils at ovicidal concentrations during July and August has resulted in an unusual type of storage scald of apples in West Virginia. This relationship was suspected in 1944–1945 and was confirmed by experiments in 1945 and 1946. The "oil scald" appears in storage as small, sharply delimited, circular hydrotic spots. They are centered around lenticels and are small, usually two or three millimeters in diameter or rarely as large as five millimeters in diameter. Larger lesions are the result of coalescence of smaller single layers of hypoderm which are plasmolyzed and discolored. The light brown color of the dead cells is modified by the color of the fruit: the color of the lesions varies widely dependent on whether the spots are on red, green, or yellow portions of the apple. The severity of the "oil scald" increases during the late storage period; but it is abundant long before normal breakdown of the fruit occurs.

A wilt inducing polysaccharide from Fusarium solani f. eumartii. THOMAS, C. A. A wilt-inducing substance produced in liquid cultures of *Fusarium solani f. eumartii* was found to be non-dialyzable, not filterable through bacterial filters, soluble in water, and relatively insoluble in most organic solvents. The substance was precipitated from non-dialyzed culture filtrates by the addition of 2 volumes of ethyl alcohol. Precipitation with alcohol from dialyzed filtrates was effected only after concentration of the filtrates. The wilt-inducing substance was thermostable in neutral, aqueous solution and labile in strong acid solution. It was partially to completely inactivated by heat in non-dialyzed filtrates at pH 7.5 and in sterile Richard's solution at pH 7.5 but not in Richard's solution at pH 5.9. It appeared that the substance was removed with phosphates precipitated by heating the alkaline, nutrient solution. Several qualitative chemical tests indicated that the toxic substance was a polysaccharide. Hydrolysis of the polysaccharide resulted in loss of wilt-inducing activity. Amount of polysaccharide was correlated with wilt-inducing activity. Tomato cuttings placed in solutions of the toxic substance wilted primarily their stems. Wilted cuttings did not recover when placed in distilled water unless sections one-half inch or more in length were cut from the basal part of the stem.

Mutants of Agaricus campestris induced by uranium salts. WAHL, I. Two distinctly new mycelial types of *Agaricus campestris* appeared in a monosporous culture of the cream brown variety growing on potato-dextrose agar containing uranium nitrate, $UO_2 (NO_3)_2 \cdot 6H_2O$, at the rate 0.1 to 1.0 gram per liter. One variant was white, fluffy, and grew rapidly, producing a mycelial mat with dry weight 5 to 7 times greater than

that of the controls on potato-dextrose agar plus $NaNO_3$. These characters remained constant in subcultures on potato-dextrose agar plus $NaNO_3$, except for several reversions to the original form. Spawn of this variant readily produced white fruiting bodies instead of the original cream-brown, and preliminary experiments indicate that it produces mushrooms earlier than the parental type, although more data are needed. Basidiospores of the white mushroom germinated readily and produced a fluffy mycelial culture. Colonies of the second variant induced by uranium nitrate are flat, pinkish, and distinctly zonate. This variant also yielded white fruiting bodies. Similar cultural mutants were induced by uranyl sulfate, $UO_2 \cdot SO_4 \cdot 3H_2O$, and uranium acetate, $UO_2 (C_2H_3O_2)_2 \cdot 2H_2O$.

Resistance in cucumber to scab. WALKER, J. C. Scab (*Cladosporium cucumerinum* (Ell. & Arth)) is the most destructive disease of cucumber in the important pickle-growing area of northeastern Wisconsin. Maine 2 variety has been highly resistant during the three seasons, 1946–1948. The optimum for disease development is about 17° C. and as the temperature increases above this the lesions tend to be corked off with little damage. Maine 2 maintained high resistance in the greenhouse under optimum conditions although infection was not precluded and watersoaked lesions appeared on the youngest leaves. Resistance was completely dominant in crosses with Chicago Pickling, National Pickling, and Stays Green, and segregation in the F_2 at the ratio of 3 resistant to 1 susceptible occurred when optimum temperature was maintained, while at higher temperatures a deficiency in the susceptible class was noted. Since Maine 2 is not a satisfactory type in the area mentioned, a program of back-crossing with standard pickle types is being pursued.

A device for planting root-rot organisms with maize seed. WERNHAM, C. C. AND R. E. PATTERSON. The device is a modification of the American standard hand drop planter. To model No. 61 a Sears Roebuck fertilizer attachment is added; model No. F 61 is rebuilt. Both models are redesigned to drop inoculum from the fertilizer attachment on top of the seed. Seed plus inoculum is planted 7–10 days early to invite a cool wet soil environment. Maize cultures consisted of 77 S_1 ears in 142 rows and 142 F_2 ears in 239 rows with bulk open pollinated corn every eleventh row serving as checks. In each row 57–60 kernels were planted. Results of 1948 are given on surviving plants in classes 0, 1–10, 11–20, etc. The open pollinated material had a higher survival value and a narrower class distribution. The root rot organisms are cultured on cooked grain and are dried before using. One bushel of inoculum will plant 10,000 hills with the planter plate half open. The device appears to be applicable to root-rot inoculations with other large seeded plants e. g. beans, cucurbits, cotton.

Production of mutants in Glomerella as influenced by age of culture. WHEELER, H. E. AND S. J. P. CHILTON. Previous studies have shown that ascigerous isolates of *Glomerella* of the Plus A type produce a large number of minus mutants, while those of the Plus B type although similar in appearance produce few if any. A comparative study of the effect of age of cultures on the production of minus mutants by Plus A and Plus B strains was made with dilution cultures using ascospores. Plus A isolates from wild morning glory and soybean behaved similarly. Only a few minus mutants appeared when cultures 10–15 days old were used but they appeared in increasing numbers as the cultures aged up to 60 days when about half of the colonies were of the minus type. Plus B isolates produced no minus cultures until 30 days old, the number then increased gradually with age but remained very low. Mutations from Plus to Minus apparently are associated with changes at a locus ''B''. The frequency of mutation at this locus was approximately 1 : 1000 from a 45 day old culture of a nonperithecial conidial strain obtained from a Plus A isolate and 1 : 10,000 from a similar conidial strain from a Plus B isolate.

Phytopathological observations in the Low Countries. WHEELER, WILLIS H.

The effect of various diluents on the infectivity of the potato X virus. WILKINSON, R. E. AND A. FRANK ROSS. *Gomphrena globosa* L. was used as a local-lesion test plant to measure the effect of various diluents on the infectivity of the potato X virus (*Annulus dubius* H.) in plant juice. Dilutions were made just prior to inoculation. More lesions were produced with inoculum diluted with distilled water than with that diluted with phosphate or borate buffers tested over a wide range of concentrations and pH values. Several amino acid buffers appeared to have some advantage over water for use as diluents. Inoculum diluted with 0.05 M histidine or lysine buffers at pH 7.5 produced about 36 per cent more lesions than did that diluted with water. Triethanolamine oleate, a wetting agent, at concentrations of about 0.06 per cent increased the infec-

tivity of the inoculum about 50 per cent over that of inoculum diluted with water. Higher concentrations of this and other wetting agents reduced the infectivity of the inoculum, especially when the plant tissue was frozen before the juice was extracted.

Electron micrographs of tobacco mosaic virus in crude expressed sap. WILLIAMS, ROBLEY C. AND RUSSELL L. STEERE. Electron micrographs of rod-like tobacco mosaic virus have usually been obtained from highly purified material, although some pictures have been published of the virus photographed in crude sap treated only by dilution with distilled water. It has recently been possible to photograph the virus (a wild strain grown in White Burley tobacco plants) in undiluted, untreated crude sap, which was allowed to dry as a thin film on the specimen screens of the electron microscope. The virus then appears in large bundles or plaques a few microns in length and a micron or so in breadth. The plaques are found to be larger as the age of the infection increases. Slight dilution or washing with distilled water of either the crude sap or the dried preparation on the microscope screen causes the bundles to break up into the characteristic individual rods heretofore photographed. The possible origin and identification of the bundles will be discussed.

Tomato anthracnose control in 1948. WILSON, J. D. AND H. A. RUNNELS. In an experiment on the influence of timing of spray applications on control, in which schedules were begun at 10-day intervals from June 10 to August 19 and ended 60 days later, it was found that beginning on July 10 or 20 was most effective. Fungicide comparisons (30 or more materials) again indicated that zinc dimethyl dithiocarbamate (Zerlate) still ranks at or near the top for anthracnose control. A test of eradicant sprays applied to the soil just before the plants fell over indicated that at least two of the materials tested may be expected to give some control of later fruit infection. The addition of various stickers, etc., to Zerlate showed little increase in control over the 2–100 formulation. A further comparison of numerous varieties, hybrids, etc., still failed to uncover any marked resistance to the disease, but considerable tolerance was exhibited by some lines. Another experiment, in which the tomato fruits were observed at 2- and 4-day intervals after picking, indicated considerable variation in breakdown after picking. Further tests with trellises indicated that keeping the fruits off the soil is more effective than any spray program in preventing infection.

Verticillium wilt of avocados. ZENTMYER, GEORGE A. A severe wilt and dieback of avocado trees (*Persea americana*) was identified during 1947–1948 as caused by *Verticillium albo-atrum* Rke. and Berth. The fungus was isolated from trees of the Fuerte variety (hybrid Mexican × Guatemalan), a budded Guatemalan variety (Anaheim), and Guatemalan seedlings, under conditions of natural infection. Affected trees were found on both Mexican and Guatemalan root stocks. Trees first show a slight wilting of the leaves, then leaves rapidly die, turn brown, and remain attached to the tree. Vascular discoloration is brown and is usually extensive from roots to affected terminal branches. Some dieback occurs, then new shoots usually appear following new xylem formation. Trees often "recover" completely, but may wilt again in subsequent years. Mexican and Guatemalan seedling avocados, inoculated by dipping the roots in a suspension of spores and mycelium, showed wilting and leaf necrosis in two weeks at soil temperatures of 15°, 20°, 25°, and 30° C., but not at 35° C. The isolate from avocado also infected tomato. Scattered diseased trees were found in all of the commercial avocado districts of California. Some of the affected groves were previously planted to tomatoes. The disease apparently was previously termed "collapse" or "asphyxiation" and attributed to excess moisture in the soil.

Soil fumigants for control of Phytophthora root rots. ZENTMYER, GEORGE A. AND L. J. KLOTZ. Several soil fumigants and fungicides were tested against two species of *Phytophthora: P. cinnamomi* causing root rot ("decline") of avocado trees, and *P. citrophthora*, cause of citrus brown rot and gummosis. The 1,3-dichloropropene, 1,2-dichloropropane complex (Dowfume N, Shell D-D) killed both fungi in inoculated soil in laboratory and greenhouse tests when injected at dosages of 50 gal./acre foot or over. 1,3-Dichloropropene is the toxic fraction; its toxicity to the fungi closely paralleled that of the complex. 1,2-Dichloropropane was ineffective as a fungicide. The normal soil microflora was not eradicated at dosages of N or D-D lethal to *Phytophthora*. In greenhouse and extensive field trials, injection of soil with Dowfume N or Shell D-D at 60 gal./acre or above has resulted in good control of avocado root rot when infested soil was treated, then replanted to avocados. Materials were applied in field trials with weed guns and tractor-drawn mechanical applicators (the latter in cooperative tests with Dow Chemical Co.). In laboratory and greenhouse tests, fumigating soil with

chloropicrin and methyl bromide and applying solutions of Dithane (disodium ethylene bisdithiocarbamate) have also killed the two fungi, although Dithane did not penetrate the soil adequately. Ethylene dibromide was fungicidal only at dosages of 100 gal./acre foot or over.

Symptoms and resistances of crop plants to charcoal rot and ashy stem blight. YOUNG, P. A. *Macrophomina phaseoli (Sclerotium bataticola)* causes gray to white bark on the stems of some legumes and other hosts. It causes rotting and hollowing of stems and tap roots, rotting of fruits, and wilting of leaves. Sclerotia color the diseased internal tissues gray or black. Pycnidia grow in the bark of legume stems. Degree of resistance is indicated by numbers: practically immune, 0; resistant, 1; moderately susceptible, 2; and very susceptible, 3. Resistance of various crops is as follows: *Crotalaria spectabilis* (late), 3; *C. spectabilis* (early), 2; *C. juncea*, 3; *C. mucronata* (Giant Striata), 1; *C. retusa*, 1; *Phaseolus vulgaris*, 3; *P. polystachys*, 0; *Vigna sinensis* (Iron), 1; *V. sinensis* (Purplehull), 3; Velvet bean and Mung bean, 2; Lima bean and Soybean (Ogden), 3; *Sesbania macrocarpa*, 3; Hubam sweet clover, 2; Guar, 0; Lespedeza, 3; cantaloupe, watermelon, and pumpkin, 3; sweet potato (stems), 1; cotton (upland), 0 to 2; okra, 2; tomato, 1; pepper (California Wonder), 2; pepper (Pimento), 0; potato, 2; asparagus, 1; turnip, 2; radish, 3; cabbage, 1; zinnia, 3; *Cosmos sulphureus*, 2; corn, 3; sorghum, 0 to 3; Sweet sudan grass, 1; German millet, 0; and Johnson grass, 0.

ANTHONY BERG
1888–1948

C . R . O R T O N

Anthony Berg, one of several children of Joseph and Barbara Berg, was born at Bloomer, Wisconsin, October 3, 1888. He received his early education in public schools at Bloomer, and entered the University of Wisconsin in 1908, from which he graduated with the Bachelor of Science degree in June, 1912. His interest in phytopathology developed under the late Professor L. R. Jones, and led him to remain another year for graduate work.

He completed the residence requirements for the Master's degree and accepted a position in March, 1913, as Assistant Plant Pathologist with Dr. N. J. Giddings at the West Virginia Agricultural Experiment Station, where he remained until his sudden death with a heart attack on February 15, 1948.

Mr. Berg completed the requirements and received the Master's degree from the University of Wisconsin in 1922. He spent the years 1922–24 at the University of Wisconsin in graduate work and completed all the requirements for the Ph.D. except the thesis. Returning to West Virginia University in 1924, he continued his duties there, and, in 1925, was made Associate Plant Pathologist in the Agricultural Experiment Station.

Berg's early work in West Virginia was divided between apple-cedar rust with Dr. N. J. Giddings and his own research subject, *Phytophthora infestans* on potato and tomato, through which he first became nationally recognized. In connection with the cedar rust investigations, he discovered a rust-resistant red cedar, *Juniperus virginiana*, which bears his name.

In 1929, Berg became interested in a disease of the apple tree commonly known as "measles," which was reported from the Kanawha Valley and other parts in West Virginia, as well as other States. His studies soon led to the conclusion that more than one specific disease was masquerading under the term "measles". He first isolated and described the fungus causing a new disease which he named "black pox" as distinguished from the Arkansas disease described by Hewitt, and still another confused but widely distributed bark disease of the apple which he described as "internal bark necrosis". This last subject engrossed his attention until his death, when he was preparing a comprehensive paper for this and related bark diseases of apple.

He was married January 25, 1937, to Genevieve Clulo, who was Assistant Professor in the University and Assistant Plant Pathologist in the Experiment Station. She had worked with Berg on the measles problem and, after marriage, continued her studies on the anatomical and physiological features of the disease. Fortunately, through her long interest, she is carrying on these studies and bringing the results to publication.

ANTHONY BERG
1888–1948

During these years, Mr. Berg spent much of his leisure time in collecting and breeding Azaleas and Rhododendrons. He brought together at Morgantown practically all the native and many of the introduced species of Azalea, a collection which is hardly matched in America. Plans are laid for the University to take over and maintain this valuable collection for scientific and educational use.

Garden clubs and nursery organizations sought Berg's services and knowledge on Azaleas and Rhododendron. He was a frequent speaker before these organizations. He was always most generous in giving advice when requested, but would never venture beyond his personal experiences which had proved to be sound.

Mr. Berg was a member of the American Phytopathological Society, the West Virginia Academy of Science, the American Association for the Advancement of Science, and Sigma Xi. He was survived by his wife, Genevieve Clulo Berg, and daughter Rita Ann, and a sister Kate and brother George, both from Bloomer, Wisconsin. His mother passed away April 15 at the age of 97 years, just two months after his death.

His colleagues have lost a loyal and valued friend who was always sympathetic and helpful in every way. "Tony", as his close friends called him, will be remembered always as a quiet, kindly person who was generous to a fault, and ever ready to help in any emergency. Phytopathology has lost, in Anthony Berg, a skillful and thoroughly trustworthy scientist who made several noteworthy contributions to science.

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AN ISOLATE OF PORIA XANTHA ON MEDIA CONTAINING COPPER

RAY R. HIRT

Poria xantha (Fr. ex Lind) Cooke is an important wood decay fungus. An extensive survey of decay in wooden buildings in central New York State, conducted by the Department of Forest Botany and Pathology of The New York State College of Forestry, has shown this fungus to be associated frequently with decay of wood in houses and industrial buildings. *Poria xantha* also is one of two fungi that has been frequently isolated from decaying timbers taken from wooden boats in service on inland waters (2).

This fungus has been used also as a test fungus to evaluate the effectiveness of certain wood preservatives suggested for controlling decay in wooden boats (5).

Hartley (3) has pointed out that it is futile to draw general conclusions from tests with one fungus under one set of conditions. This may be due in part to the fact that some fungi react differently to different wood preservatives. For instance, *Poria xantha* is stated to be somewhat tolerant of sodium fluoride (1) and copper at low concentrations (5); and *Lentinus lepideus* Fr. tolerant to creosote and pentachlorophenol (4). It is necessary to be familiar with the different responses of wood decay fungi to wood preservatives if correct interpretations are to be made in evaluating the preservatives. In standardizing tests it is also important to recognize these different responses in order to select the proper test fungi.

Poria xantha has been grown in culture under various conditions at The New York State College of Forestry. While incubating the fungus on standard malt agar containing different concentrations of copper naphthenate, a peculiar pattern of cultural behavior was observed. The phenomenon was considered sufficiently important to be brought to the attention of those individuals working with wood decay fungi.

THE FUNGUS

The fungus used for this study was *Poria xantha,* Culture No. 213a of the Division of Forest Pathology, Beltsville, Maryland. It was isolated by Dr. Ross Davidson from decaying mahogany wood collected by the writer in 1942, from the transom planking of a fresh-water cabin cruiser. Sporophores and mycelium of the fungus were present on several of the transom planks and adjoining hull planks, but Cultures No. 213a and No. 213 were derived from the decaying wood.

In stock cultures the fungus was grown on standard malt extract agar. To observe the reaction of the fungus to copper in the nutrient agar, copper

naphthenate was added to malt agar in proper quantities to supply the desired amount of copper metal. Standard malt agar containing the following concentrations of copper metal on the basis of weight was prepared: 0.00001 per cent; 0.0001 per cent; 0.001 per cent; 0.01 per cent; and 0.1 per cent. Ten Petri dishes of each copper concentration were poured and also 10 controls without copper. A technique was devised which insured a uniform distribution of the copper metal throughout the agar. Plantings of the fungus taken from a single culture were made in each of the dishes. The cultures were incubated at approximately 28° C.

<center>RESULTS</center>

In all of the control cultures on malt agar as well as in all the dishes containing agar with 0.00001 per cent, 0.0001 per cent, and 0.001 per cent concentrations of copper metal the fungus grew in its characteristic manner, except for differences in rate. In agar with 0.01 per cent concentration of copper metal the fungus grew in only 2 dishes, and in only one dish that contained a concentration of 0.1 per cent copper. The original inoculum from each of the 17 Petri dishes in which the fungus had failed to grow was removed after 16 days and placed on fresh malt agar without copper. No growth occurred, hence it is evident that the mycelium had been killed in 17 of the 20 cultures containing the two highest concentrations of copper metal.

The daily average rates of diameter increase of the surviving cultures from the sixth to the tenth days after establishment on the culture media were: no copper, 0.95 cm.; 0.00001 per cent copper, 0.91 cm.; 0.0001 per cent copper, 0.80 cm.; 0.001 per cent copper, 0.77 cm.; 0.01 per cent copper, 0.40 cm.; and 0.1 per cent copper, 0.19 cm. The copper had an adverse effect upon the growth rate of the fungus.

Surviving cultures on malt agar containing 0.01 per cent copper metal. The visual appearance of the mycelium did not vary noticeably from that in the control cultures. Mycelia taken from the two cultures that grew on malt agar containing 0.01 per cent copper metal were subcultured on similar nutrient media where they continued to grow. However, plantings from these cultures never succeeded in becoming established on malt agar with 0.1 per cent copper. For convenience here this mycelium is designated as Culture No. 213–50.

Surviving culture on malt agar containing 0.1 per cent copper metal. In the young culture the mycelium formed approximately a solid circle on the agar. As the culture aged, the growth gradually assumed a less regular shape with several sectors growing relatively slowly. The faster growing area of the mycelium had developed numerous radiating strands of yellow compact hyphae. The visual appearance of the slow-growing and rapid-growing sectors of mycelium was sufficiently distinct to attract the attention of a close observer. Subcultures were made from these two different

sectors and hereafter are designated as: Culture No. 213–501, from the area with the faster growing mycelium containing strands; Culture No. 213–503, from the slower growing uniform mycelium between the areas with strands.

Culture No. 213–503 could be subcultured usually on malt agar containing 0.01 per cent copper. However, it could not be re-established on nutrient agar with 0.1 per cent copper metal, even though this culture was originally isolated from such a substratum. Cultures No. 213–50 and No. 213–503 seem to be alike in all respects.

Culture No. 213–501, when established, grows vigorously on malt agar containing 0.01 per cent copper and 0.1 per cent copper metal. However, if it is transferred to and grown upon malt agar without copper, only occa-
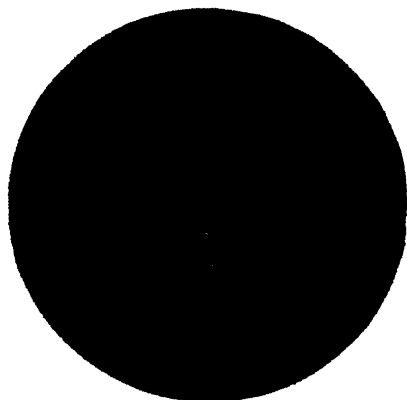
FIG. 1.   A young culture of *Poria xantha* No. 213–501 on malt agar containing sufficient copper naphthenate to give a copper content of 0.1 per cent copper by weight.   The mycelium is surrounded by two zones of faded agar: *a*, the darker zone; *b*, the lighter zone.   The unaffected dark green agar is designated *c*.

sional subcultures are again successful on agar with 0.1 per cent copper. On agar with 0.01 per cent copper, growth is established more slowly when the inoculum is taken from malt agar without copper than with copper. Subcultures of the fungus direct from agar containing 0.1 per cent copper grow rather vigorously on malt agar containing either of the concentrations of copper metal, but more rapidly with the lower concentration (Fig. 2).

Malt agar containing 0.1 per cent copper metal is deep green. In successful cultures, approximately 48 hours after planting the mycelium of culture No. 213–501 on the agar, the green color of the agar begins to fade adjacent to the inoculum. This light colored region becomes wider and another narrow band of almost colorless agar appears outside the first faded area. The mycelium then begins to grow out from the inoculum (Fig. 1). After that, the cultures appear as a solid center of yellowish mycelium surrounded by two concentric bands of light colored agar enclosed within the deep green of the unaffected nutrient copper-agar. As

the mycelium grows the bands of light colored agar enlarge until they reach the walls of the Petri dishes. Apparently the faded bands of agar around the mycelium are fields of chemical activity associated with invasion by the fungus mycelium.

Solid cylinders of agar from the faded areas about the mycelium growing on 0.1 per cent copper agar were removed and placed on sterile 0.1 per cent copper agar. Mycelium from cultures No. 213a, No. 213–50, No. 213–501, and No. 213–503 were placed on the surface of these agar cylinders. Only the mycelium of culture No. 213–501 subcultured from agar with 0.01 and 0 1 per cent copper continued to grow. Apparently the loss
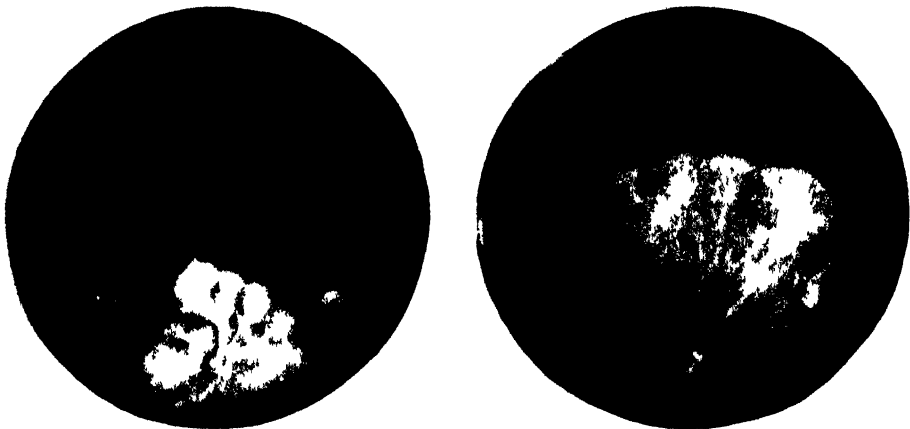
FIG. 2. Left: *Poria xantha* No. 213–501 growing on nutrient agar containing 0.1 per cent copper. Right: On agar containing 0.01 per cent copper. The unsuccessful plantings in each Petri dish are from the other cultures mentioned in the text. Cultures are the same age.

of the green color from the copper-agar due to the activity of mycelium No. 213–501, did not cause the agar to become a favorable substratum for the other 3 cultures.

As previously stated, on the agar with the high concentration of copper the fungus has a tendency to develop very compact rhizomorph-like strands of yellow mycelium (Fig. 2). It is from these strands that the most active growth occurs as the fungus spreads over the copper agar (Fig. 2, left). In subculturing the fungus onto new plates of nutrient agar with the highest concentrations of copper, more subcultures were successful if pieces of the strands were used rather than the more uniform mycelial growth between the strands. In fact, subcultures from the uniform growth areas were rarely successful on agar with 0.1 per cent copper, and it is possible that minute strands of the mycelium may have been present in those successful inocula. Mycelia from either area became established quickly on malt extract agar lacking copper.

It is possible that the parent culture of No. 213–501 represents a transfer of a portion of a rhizomorph-like strand from culture No. 213a. On

plain malt extract agar the compact strands are much less abundant than on agar containing copper. Therefore if the strands of compact hyphae are a factor in establishing the fungus on agar containing copper, fewer inocula from plain nutrient agar would be apt to succeed on copper-containing-agar than inocula taken from cultures on agar with copper, since in the former cultures the hyphal strands are relatively less abundant. The parent of culture No. 213–501 was the only successful culture from 10 plantings of mycelia No. 213a on 0.1 per cent copper agar.

Culture No. 213–503 was taken from slow-growing mycelium of uniform appearance that developed between the mycelial areas with rhizomorph-like strands growing on agar with 0.1 per cent concentration of copper. The slower growing mycelium lacks coarse strands and it also fails to form the tough skin-textured mycelium that can be peeled from the surface of the agar, such as that associated with the production of the coarse strands. This more uniform mycelium continues to grow between the strands but definitely slower than the strands themselves. This mycelium is associated with the strands and is probably composed of hyphae growing out from them, but it remains less compacted and may have a function other than that of directly extending the fungus on difficult substrata. Since culture No. 213–503 could not and No. 213–501 could be re-established on nutrient agar containing 0.1 per cent copper, and yet both grew vigorously on plain malt agar, apparently there is a fundamental difference between the two cultures No. 213–501 and No. 213–503.

Inocula from actively growing cultures of No. 213–50, No. 213–501, No. 213–503, and the original culture of *Poria xantha* No. 213a were placed at four separate points on malt agar in each of 5 Petri dishes. As the mycelial masses increased in size no differences were visible in the separate plantings, except for a somewhat more pronounced yellow in plantings No. 213–501. No lines of demarcation occurred between the separate mycelia, and eventually they grew together giving the appearance of a single culture in each Petri dish. This indicates, but does not prove, that *Poria xantha* No. 213a originally was the mycelium of a single fungus. The hyphae from the four cultures are similar microscopically.

SUMMARY AND CONCLUSIONS

Inocula from *Poria xantha* culture No. 213a were subcultured on nutrient agar containing copper in various concentrations. Copper adversely affected the development of the mycelium causing it to grow with more difficulty as copper was increased.

On agar containing 0.1 per cent copper metal only 1 planting of 10 succeeded in growing and this culture was characterized by rhizomorph-like strands. Portions of these strands when used as inocula readily established the fungus on other copper-containing agars with 0.1 per cent copper or less.

The mycelium between the strands was uniform in appearance and slow in growth. It could not be successfully transferred to and established on nutrient agar with 0.1 per cent copper, but readily grew on malt extract agar without copper.

Apparently the more favorable the agar substratum is for the growth of *Poria xantha* culture No. 213a, the less tendency there is to produce rhizomorph-like growths, and consequently the less chance there will be of successfully establishing the fungus on unfavorable substrata such as a 0.1-per-cent copper agar. On the other hand, if the fungus can be established on a nutrient agar containing as much as 0.1 per cent copper, the fungus produces many strands of compact hyphae which serve as favorable inocula for planting on similar copper-containing agars.

Apparently, such copper tolerance as *Poria xantha* may possess is associated largely with the ability of the fungus to produce strands of compact hyphae which, en masse, are able to endure until the fungus adjusts itself to the new environment. Even then, the fungus grows much more slowly than on nutrient agar lacking copper.

Unless care is exercised to secure uniform inocula from cultures of *Poria xantha* No. 213a growing on agar, it is possible to secure widely varying results in testing its reaction to copper-containing-substrata.

The results obtained in this study emphasize the statement already cited (3), that general conclusions regarding the effectiveness of a wood preservative may be very misleading when based on a single test or a single fungus. This is especially true where agar tests are used.

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TRANSMISSION OF CHERRY YELLOWS VIRUS COMPLEX THROUGH SEEDS[1]

DONALD CATION[2]

Certification of stone fruit varieties for the control of virus diseases is of interest to both fruit growers and nurserymen. In several States nurseries complying with prescribed standards of scion wood selection and a degree of isolation from possible disease sources are given certificates for freedom from disease so far as can be determined by inspection by the regulatory agencies. Such certificates are of limited value if seedling rootstocks are carrying viruses. Because many viruses may be masked in different species and varieties so that roguing is of nominal value only, an exact knowledge of the transmission of stone fruit viruses through seeds is of immediate importance.

Hildebrand[3] observed that certified budwood did not always result in disease-free trees since widely separated trees in the nursery row were affected with yellows. This was circumstantial evidence of rootstock contamination. He also observed abnormalities in occasional seedlings of *Prunus Mahaleb* grown in nurseries. An unspecified amount of indexing from such trees gave symptoms that he considered typical of ring spot and cherry yellows.

Cochran[4] detected visual symptoms of ringspot in seedlings that he grew from *Prunus avium* and demonstrated the transmission of virus through seed by transmitting it to peach from five of the six "Mazzard" seedlings tested but obtained no symptoms from six seedlings having no visual symptoms. His similarly grown Mahaleb seedlings from ring spot-infected trees showed no symptoms and apparently were inferred to be virus free as they were not reported indexed on peach.

METHODS USED TO DETERMINE SEED TRANSMISSION

The cherry yellows disease is considered a complex of at least two viruses, one of which gives symptoms identical to those of the ring spot virus while the other component has not been determined. Cherry yellows is not detectable in Mahaleb cherry by casual examination. It must be determined by indexing on reacting hosts such as sour cherry varieties or peach seedlings.

[3] Hildebrand, E. M. Viruses and cherry rootstocks. Amer. Nurseryman 82(7): 5–8, 18–21. 1945.

[4] Cochran, L. C. Passage of ring spot virus through "Mazzard cherry seeds." Sci. 104: 269–270. 1946.

To study the possibility of seed transmission of the cherry yellows virus components, seeds were collected in 1946 from Mahaleb sprouts growing from the roots of diseased Montmorency cherry trees. The seed was held out-of-doors in moist peat until October 1, then placed in cold storage at 40° F. for about three months. When individual seeds showed signs of sprouting they were planted in flats in the greenhouse. Only a few of approximately 1000 seedlings had any abnormal mottling or other possible symptoms. Similarly, seeds were saved and grown from a Montmorency tree considered to have typical cherry yellows symptoms.

Plant tissue from a random sampling of the normal-appearing seedlings was grafted in August, 1947, to yearling seedling peach trees growing in the field. Transmission readings were made during the first six weeks of growth in 1948, mostly during May.

Because of the limited number available, only 65 peach trees were used to index the seedlings from Mahaleb. Each peach tree received tissue grafts from three different Mahaleb seedlings. The percentage transmission thus could only be estimated. Likewise 79 peach trees were used to index 227 Montmorency seedlings.

EVIDENCE FOR SEED TRANSMISSION

The data in table 1 and in figure 1 illustrate that at least 42 of the 65 peach trees received one or more viruses resulting from inoculations with

TABLE 1.—*Transmission through seeds of Mahaleb and Montmorency cherry of component viruses concerned with the cherry yellows complex as determined by indexing on seedling peach trees*

Data recorded	Source of graft tissue	
	Mahaleb seedlings	Montmorency seedlings
Number of cherry seedlings indexed	195	227
Number of peach trees inoculated	65	79
Number of diseased peach trees:		
Necrotic ring spot[a]	20	68
Cherry yellows[b]	17	0
Indeterminate, trees killed[c]	5	2
Total	42	70
Number of peach trees with no reaction	23	9

[a] Delayed foliation, die back, later sprouts normal.
[b] Delayed foliation, die back, later sprouts rosetted.
[c] Delayed foliation and die back. Either ring spot or cherry yellows may have caused death.

tissue grafted from Mahaleb seedlings. However, as 195 Mahaleb seedlings were tested, the virus transmission was approximately 21.5 per cent. Of these, at least 20 or approximately 10 per cent transmitted ring spot virus while at least 17, or approximately 8.7 per cent transmitted the cherry yellows virus complex.

In the Montmorency determination, 79 peach trees received graft inoculations from 227 Montmorency seedlings. Of these, 9 peach trees remained normal while 68 showed symptoms typical of ring spot infection and two died from the result of virus infection. In case of death, the lack of sprouts made exact diagnosis impossible. The amount of disease transmission was at least 30 per cent. It is of interest to note that a greater number of cases of virus transmission through seed was demonstrated for Montmorency than for Mahaleb and that only the ring spot component of

FIG. 1. Results of Mahaleb indexing on peach. Trees A and B show recovery from ring spot symptoms. Tree C is dead and trees D to I show the rosetted growth typical of cherry yellows on peach   Photographed June 2, 1948.

the cherry yellows complex was involved. Examples of peach reaction to Montmorency indexing are shown in figure 2,A and B.

The possibility that the Mahaleb and Montmorency seedlings acquired infection in the greenhouse is remote, because 75 previously disease-free Montmorency trees growing in the same greenhouse were similarly indexed and transmitted no infection to peach. The infection of seedling peach in the field from outside sources is improbable because none of the many control trees showed symptoms. Therefore, the data appear to offer conclusive proof of virus transmission through seed.

In another experiment, 325 cherry trees propagated from previously determined disease-free budwood and grown on a commercial source of Mahaleb stock were indexed and found to have 9 cases of cherry yellows and 5 cases of ring spot. In all cases only one of the several to many budlings propagated from an individual tree became diseased while all other budlings propagated from the same tree indexed disease free. The probable explanation is that some of the Mahaleb stocks were infected.
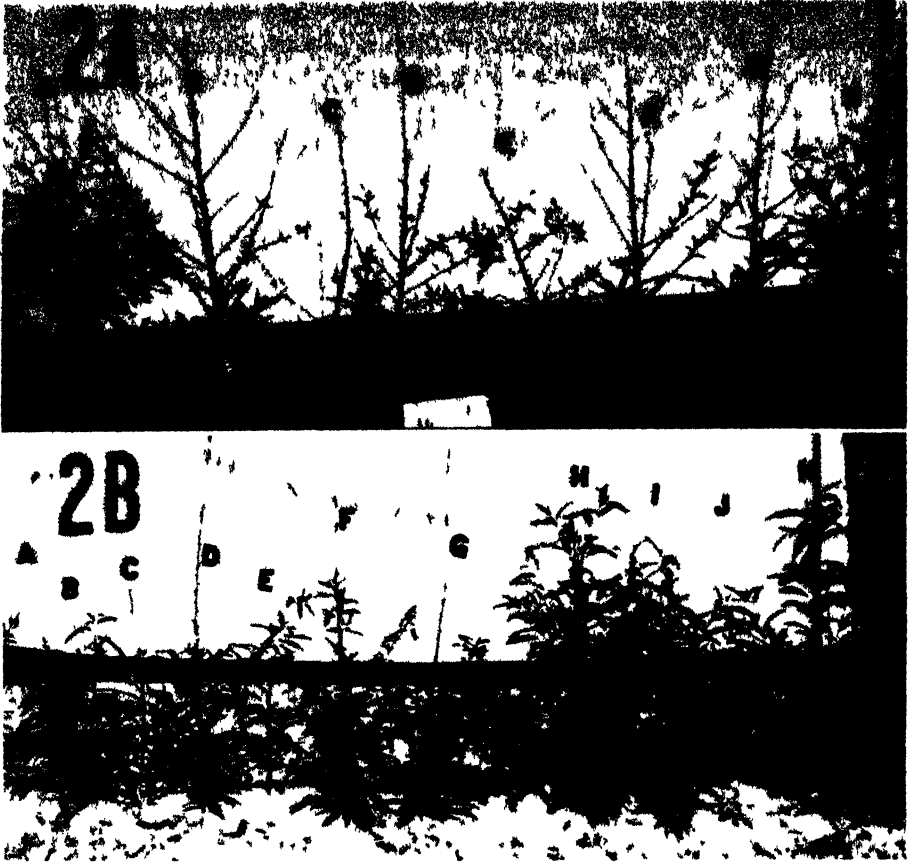
FIG 2   Results of Montmorency seedling indexing on peach   A   Trees A and H are normal, while trees B to G show the delayed foliation typical of ring spot infection   Photographed May 15, 1948   B   Trees A to G show recovery following delayed foliation while trees H to K are normal noninoculated controls   Photographed June 4, 1948

SUMMARY AND CONCLUSIONS

Mahaleb and Montmorency seeds collected from known cherry yellows-diseased trees were grown to seedling stage in the greenhouse and indexed on seedling peach in the field   It was demonstrated that at least 10 per cent of the Mahaleb seeds transmitted the ring spot virus and at least 8 7 per cent transmitted the cherry yellows complex   The cherry yellows complex was not transmitted through the seed of Montmorency in these experiments but at least 30 per cent of the seeds carried the ring spot virus

The foregoing studies are believed to offer the first detailed account of seed transmission of viruses concerned with the cherry yellows complex. They emphasize the need of a disease-free source of seedlings in programs of stone fruit virus certification, particularly for cherry understocks.

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FIELD INFECTION EXPERIMENTS WITH RACES 15A AND 15B OF PUCCINIA GRAMINIS TRITICI[1]

T. JOHNSON[2]

From the point of view of stem-rust resistance, the great merit of the Hope– and H-44–derivative wheats, now widely grown in the United States and Canada, is that they are resistant in the adult plant stage to all the physiologic races of *Puccinia graminis tritici* Erikss. & Henn. that have been prevalent in the regions in which these varieties are grown. The advent of any physiologic race of more than usual virulence towards these varieties is therefore a matter of practical importance.

The wide interest in race 15B is due to the fact that it was reported more pathogenic than other races to many rust-resistant wheats, including Hope and H-44 derivatives (2, 4). The severe infection of the latter wheats by this race appears to be the first instance in North America of breakdown of the adult-plant resistance of the Hope–H-44 type. Although race 15B has not occurred commonly in the United States or Canada in past years, there is no known reason why it should not at some future time gain wider prevalence than heretofore. In view of the possible future spread of this race, it seemed desirable to determine as accurately as possible, under field conditions, its virulence towards the stem-rust resistant wheats now grown in Canada and, particularly, to compare its pathogenicity with that of race 15A, which has been present in this country for many years. It was also thought desirable to acquire a more precise knowledge of the reaction to these two rust biotypes of wheats now in use as breeding material, such as the Kenya wheats, McMurachy, and Red Egyptian. Tests in the greenhouse at Winnipeg, in 1945, had indicated that race 15B possessed greater pathogenicity than race 15A towards Regent, Redman, Thatcher, and certain other varieties; but, as greenhouse tests can not be regarded as adequate indications of field reaction, it was felt that decisive information of the kind desired could be secured only in field tests. Accordingly, tests were carried out with a group of varieties listed in table 1 during the summers of 1945, 1946, and 1947, in small field plots inoculated with pure cultures of certain accessions of races 15A and 15B. These plots, composed of 5-foot rows surrounded by guard rows of a susceptible wheat, were sown at a distance from each other and from any other plots subjected to artificially produced stem-rust infestation. The plots were sown as early in the spring as practicable so as to escape natural stem-rust infection in so far as possible.

Rust inoculation was performed at time of heading by the method described by Cherewick (1). Briefly, this consisted in dusting each plot as

uniformly as possible with a spore-talc mixture containing pure inoculum of the physiologic race employed. By using a large supply of inoculum, it was possible to distribute spores evenly over the plot in large enough quantity to produce heavy, uniform infection in minimum time. By this procedure it was possible to take rust readings on these plots before stem-rust infection from other sources became general.

EXPERIMENTAL RESULTS

The data derived from the plot experiments are summarized in table 1

TABLE 1.—*Percentage infection[a] produced by different accessions of races 15A and 15B of stem rust and by mixtures of races on certain wheat varieties*

Wheat variety	1945 Race and source[b]			1946 Race and source			1947 Race and source					
	Mixture	15A (C)	15B (M)	Mixture	15A (W)	15B (M)	15A (C)	15A (W)	15A (N)	15A (SC)	15B (M)	15B (K)
Vernal		18	55	15	5	70	5	5	15	tr	23	48
Kenya, R. L. 1373	tr	tr	1	0	5	20	tr	tr	tr	1	30	8
Kenya × Gular												
8869							1	1	8	tr	15	30
8871							tr	tr	1	tr	18	35
8872							1	1	6	tr	5	35
Red Egyptian	tr	tr	4	tr	tr	25	tr	tr	tr	tr	20	4
McMurachy	tr	tr	13	tr	tr	20	tr	tr	tr	tr	28	10
R. L. 2327c				tr	10	55	6	5	3	1	30	38
Thatcher	8	25	73	40	10	80	13	18	15	2	48	68
Rival	1	28	63	60	tr	70						
Regent	tr	2	50	15	10	75						
Redman				20	10	75	15	8	5	3	60	70
Hope	tr						1	5	5	5	35	35
Iumillo	tr	tr	20	tr	tr	20	0	0	0	0	1	2
Carleton				50	75	70						
Stewart				50	60	75						
T. timopheevi		tr	4	tr	0	5	tr	tr	0	0	tr	4
Garnet				85	75	75	90	88	88	90	70	80
Marquis	88						85	85	88	85	75	83

[a] Figures for 1945 and 1947 are averages of readings of two plots.
[b] The mixture of races did not contain race 15B. Sources of accessions are designated as follows: (C)—Cullen, Sask.; (M)—University of Minnesota; (W)—barberry, Winnipeg, Man.; (N)—Normandin, Que.; (SC)—Swift Current, Sask.; (K)—Killarney, Man.
[c] A hybrid line derived from the cross (McMurachy × Exchange) v Redman.

in the form of percentage of infection estimated according to the Cobb scale as modified by the U. S. Department of Agriculture.

Four accessions of race 15A and two of 15B were used. The first accession of race 15B was kindly supplied by Dr. E. C. Stakman, Department of Plant Pathology, University of Minnesota, in the spring of 1945; the second was collected at Killarney, Manitoba, in 1946. The four accessions of race 15A were all of Canadian origin. All of the rust cultures employed had been identified as either race 15A or 15B in greenhouse tests prior to their use in the field experiments.

To compare the severity of infection produced by race 15B with infection produced by a mixture of stem-rust races, inoculations were made simultaneously, except in 1947, with a race-mixture containing nearly forty physiologic races including race 15A but not race 15B.

1945 experiments—Only one accession of race 15A (collected at Cullen, Saskatchewan) and one of 15B (Minnesota accession) were available in 1945. Averages of rust readings of two separate replicates of each of the varieties included in the plots are given in table 1. A comparison of the percentages recorded for the two biotypes shows race 15B to be much more virulent than race 15A towards the varieties Vernal, Thatcher, Rival, Regent, McMurachy, and Iumillo; and somewhat more so towards Red Egyptian and *Triticum timopheevi*.

No yield data were taken but 1000-kernel weights showed that race 15B caused much more shrinkage of kernels than did race 15A in the varieties Rival, Regent, and Thatcher.

The results obtained with a mixture of physiologic races containing all available races except 15B resembled those obtained with race 15A, except that the latter rusted the variety Rival more heavily.

1946 experiments—In 1946, the accession of race 15B from the University of Minnesota was compared, in similar field plot experiments, with a culture of race 15A isolated from aecia collected on a naturally infected barberry at Winnipeg. Two separate plots were infected with each biotype but, owing to the accidental destruction of one set of plots, only one reading on each wheat variety was secured for each biotype. For comparison with the two biotypes, another plot was infected with a mixture of stem-rust races other than 15B.

The infection results for the two biotypes were strikingly different. On the varieties Vernal, Thatcher. Rival, Regent, and Redman, race 15B produced infection recorded as 70 per cent or more, whereas the infection produced by race 15A did not exceed 10 per cent. That these differences were due to the greater virulence of race 15B towards these varieties and were not the result of a generally heavier infection in the plot inoculated by race 15B is shown by the fact that race 15A produced about the same amount of infection as 15B on the durums Carleton and Stewart (60–75 per cent) and on the susceptible wheat Garnet (75 per cent).

The infection results with the mixture of stem-rust races resembled those obtained with race 15A, except that the race-mixture produced heavier infection on Rival and Thatcher.

1947 experiments—In 1947, field plots were infected by two different accessions of race 15B (one from Minnesota and another collected at Killarney, Manitoba, in 1946) and by four Canadian accessions of race 15A.

The infections recorded in table 1 show clearly that both accessions of race 15B were decidedly more pathogenic than any of the cultures of race 15A towards all the varieties tested except the susceptible wheats Garnet and Marquis and possibly the highly resistant *Triticum timopheevi*.

In these tests there are some indications of pathogenic differences between the two accessions of race 15B. The three strains of Kenya × Gular included in the tests were attacked more heavily by the Killarney accession than by the one obtained from Minnesota—a result in agreement with seedling tests. On the contrary, the varieties Kenya, Red Egyptian, and McMurachy were attacked more heavily by the Minnesota accession.

The four accessions of race 15A did not differ greatly in the percentages of infection recorded except that the Swift Current culture produced generally less infection than the others.

DISCUSSION

A study of the data in table 1 will show that the six accessions of wheat stem rust used in the field-plot experiments fall into two groups as to amount of infection caused on the resistant wheats. One of these groups is composed of the four cultures identified in seedling tests in the greenhouse as race 15A; the other is made up of the two cultures identified as race 15B. The most satisfactory of the various seedling infection tests tried thus far for differentiating the two biotypes is the reaction of three hybrid lines of the cross Kenya × Gular. These lines were highly resistant to the cultures designated as race 15A and moderately susceptible to those designated as 15B. The two groups of cultures showed other, though less striking, pathogenic differences, such as a somewhat greater virulence on the part of the two 15B cultures towards seedlings of the varieties Kota, Rival, and Fronteira. The infection results obtained in the field make it clear that the pathogenic characteristics of these rust cultures on seedling plants are also expressed to a considerable degree on adult plants of the same varieties growing in the field. In the varieties Hope, Regent, and Redman, there appears to be no seedling resistance to either biotype of race 15 but, as shown in the field experiments, there is a certain degree of adult-plant resistance to race 15A but not to race 15B.

Although the cultures studied are here designated as either race 15A or race 15B, there are probably slight pathogenic differences among individual cultures of each group. The Killarney accession of the B biotype is somewhat more pathogenic to Kenya × Gular in both the seedling and the adult-plant stages than that from Minnesota and it is possibly slightly more pathogenic to *Triticum timopheevi*. In this connection it is worth noting that Vallega and Favret (5) have recently described a culture of race 15, collected in Argentina, to which *T. timopheevi* was fully susceptible in greenhouse tests.

The results of the field experiments support the conclusion (2) that race 15B is potentially dangerous to the rust resistant wheat varieties now grown in Canada and the United States and they suggest further that this biotype may, under conditions prevailing at Winnipeg, do moderate damage to varieties with stem-rust resistance of the kind possessed by Kenya R.L. 1373, McMurachy, and Red Egyptian.

The amount of infection produced on these three varieties by race 15B (Minnesota accession) was not very consistent from year to year. Kenya R.L. 1373, for example, bore only trace or 1 per cent infection in 1945, but had 20 per cent infection in 1946 and 30 per cent in 1947. That this inconsistency is the result of environmental influences is likely as it has been shown (3) that the stem-rust reaction of both Kenya R.L. 1373 and McMurachy is influenced by temperature. It is worth noting that these varieties react very differently to stem rust in different parts of the world. In Canada they have shown, apart from their reaction to 15B, a high degree of stem-rust resistance. In Kenya Colony, East Africa, they have been classed as stem-rust susceptible wheats,[a] having proved considerably more susceptible than the II-44–derivative Regent.

The amount of infection caused by the two accessions of race 15B used in these experiments compares rather closely with that reported by Hart (2) for a mixture of physiologic races in which the B biotype evidently played a prominent, though not an accurately determinable, part. In the present experiments, 15B was not compared with such a mixture of races but comparisons were made in 1945 and 1946 of race 15B and a mixture of all other available races, including 15A (Table 1). The infection produced by this race-mixture is comparable with the infections usually produced by such mixtures in the artificially-produced stem-rust epidemics on field plots at Winnipeg, although in 1946 it was somewhat heavier than usual on Rival and Thatcher. Race 15B differed from the race-mixture by causing a much heavier infection on Vernal, Kenya, Red Egyptian, McMurachy, R.L. 2327, Regent, Redman, and Iumillo.

Although the B biotype of race 15 has apparently shown little or no increase in prevalence since its discovery in the United States, there is a possibility that it may sooner or later come into prominence. This possibility should be kept in mind by pathologists and breeders concerned with wheat breeding for stem-rust resistance and any future breeding program should include a search for wheats with a specific resistance to this race.

SUMMARY

Four different stem-rust cultures identified by means of seedling infection tests as race 15A and two cultures identified as race 15B were compared for pathogenicity towards a group of wheat varieties in small field plots. Race 15B was decidedly more virulent than race 15A on the stem-rust-resistant common wheats now grown in Canada and on a number of other wheats generally regarded as possessing high stem-rust resistance.

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THE TOXICITY OF POLYSACCHARIDES AND OTHER LARGE MOLECULES TO TOMATO CUTTINGS[1]

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INTRODUCTION

Previous fractionation (10) of a filtrate from cultures of the crown-gall organism, *Phytomonas tumefaciens* (Smith and Townsend) Bergey *et al.*, yielded a wilt-inducing, alcohol-insoluble substance. This consisted largely of the polysaccharide previously isolated both from a virulent (13) and from an attenuated culture (8). The main wilt-inducing component secured by purification of this fraction was a glucosan.

This glucosan contained no specific functional groups thought capable of imparting this unique activity to the molecule. The question arose whether other polysaccharides and also different types of substances of medium molecular weight might induce wilting. Certain of these have been examined for such activity in tomato cuttings. The results both of these tests and of some experiments on the mechanism by which such molecules induce wilting are presented in this paper. Abstracts covering part of this work have appeared already (9, 11).

MATERIALS AND METHODS

The gums and polysaccharides and other polymers were obtained from various sources. Preparations of glucosan from crown-gall bacteria were the same as those previously described (8). Gum from *Rhizobium trifolii* Dangeard was prepared according to the procedure of Hopkins, Peterson, and Fred (12). The polyethylene glycol and Carbowax samples were kindly supplied by the Carbide and Carbon Chemical Company; the samples of polyvinyl alcohol by the E. I. du Pont de Nemours and Company. The other substances were supplied as indicated in table 1 by various investigators to whom the authors express their grateful thanks.

The methods used both in growing the tomato plants and in testing the various preparations were the same as those previously described (10). The condition of the plants used for testing influenced the type and severity of symptoms. Young, succulent cuttings wilted more readily and showed stem and petiole flaccidity more completely than older cuttings.

Unless otherwise stated, the materials were assayed at 0.4 per cent concentration (certain of these showed activity in 0.1 per cent solution), and all samples were adjusted to pH 3.0 before the assay. For each test of a solution, 5 or more cuttings were employed. Each cutting was placed in a gradu-

ated test tube containing from 5 to 20 ml. of solution. Tests were made in a room with controlled temperature at approximately 25° C., with relative humidity at about 70 per cent, and with continuous light from a series of fluorescent lamps.

Reducing sugar was determined according to Shaffer and Somogyi (21, Reagent 50 with 5 gm. potassium iodide per liter) and to the micro-colorimetric method of Somogyi (22). Polysaccharide was estimated from the difference in the amount of reducing sugar formed upon hydrolysis with 1 N hydrochloric acid at 100° C. in 10 minutes and in 2 hours. Carbowax compounds were estimated by a modification of the Shaffer and Critchfield (20) method. In the modification the samples were precipitated, washed, digested, and the colorimetric estimations were made in the same tubes. Since the determination was made in a smaller container than that used by the above investigators, i.e., 14×128 mm. Pyrex colorimeter tubes, proportionately smaller quantities of sample and reagents were employed. Colorimetric readings were made with the Klett-Summerson photoelectric colorimeter containing a 520 mμ filter. The range of the assay is from 20 to 80 γ Carbowax per tube.

RESULTS

Activity of various polysaccharides and gums.—Aqueous solutions (0.4 per cent) of various polysaccharides and gums from higher plants and from microorganisms were tested for wilting activity in tomato cuttings. The degree of activity of each substance was estimated, when possible, from the amount of solution absorbed by the cutting and the amount of wilting or injury induced, as previously described by Hodgson *et al.* (10). This method was developed primarily to estimate the extent of wilting when such injury was confined to the leaflets. It could not be used satisfactorily when the stems and petioles particularly were affected.

Preparations from black spruce, *Betacoccus arabinosaceus* Orla-Jensen (*Leuconostoc mesenteroides*), and *Rhizobium trifolii* were assayed once since only limited quantities of the substances were available; all others were assayed at least twice.

All the preparations listed in table 1 wilted tomato cuttings. However, the types of wilting observed, varied. The preparations were divided roughly into two groups depending upon the type of wilting induced, *e.g.*, (a) those affecting primarily the leaflets leaving the stems and petioles turgid (Fig. 1, A and D) and (b) those producing a generalized wilting of the cutting, affecting particularly the petioles and to a lesser extent the stems and leaflets (Fig. 1, B and C).

Certain properties of the preparations appeared to be associated with the type of wilting observed. The substances readily soluble in water and yielding clear, non-opalescent solutions affected primarily the leaflets. Those that were less water-soluble and that yielded opalescent solutions usually affected primarily the petioles and stems.

FIG 1   A Tomato cuttings after 48 hours, left to right, in solutions of inulin, soluble starch, glucosan from crown gall bacteria, and distilled water.   The first three cuttings are injured severely, as shown in detail in D.   B As A except containing xylan, pectin, agar, and distilled water   The flagging of petioles and stems is conspicuous   However, leaflet injury is slight, as shown in detail in C.   C. Representative leaflets from a cutting in a solution of glycogen, a polysaccharide wilting petioles and stems with only slight injury to leaflets.   D. As C except from a cutting in a solution of glucosan from crown gall bacteria, a polysaccharide inducing leaflet wilting and necrosis, but with little effect on petioles and stems.

Among those preparations primarily affecting leaflets, some variation was noted in the syndrome. When the injury was mild, some substances (*e.g.*, glucosan of *Phytomonas tumefaciens* and corn syrup dextrins) induced curling and rolling of the tips and edges of the leaflets, while others (*e.g.*, inulin and soluble starch) caused a collapse of the outer portions of

TABLE 1.—*Wilting activity of polysaccharides and related substances*

Type	Source* or name	Approx. mol. wt.	Number of cuttings	Solution intake[b]	Toxic index[b]	Toxic units[b]
				Ml.		*Per gm.*
Compounds acting mainly on leaflets						
Fructosans	*Bacillus subtilis* 1		9	2.2	3.4	390
	Azotobacter indicum 1		9	1.9	2.3	330
	Inulin 2	5,000	15	3.7	4.1	280
Glucosans	Soluble starch 2	4,000	30	3.4	3.6	270
	Phytomonas tumefaciens 3	3,600	20	6.2	2.6	100
	Corn syrup dextrins 4					
	12 glucose units	2,000	16	10.7	1.7	40
	7.5 glucose units	1,200	16	11.2	1.3	30
	5.4 glucose units	1,000	16	11.9	1.2	25
Mixed	Black spruce 5		8	2.9	0.7	60
Compounds acting mainly on petioles						
Glucosans	*Betacoccus arabinosaceus* 6	2,600	5	1.2		
	Unidentified bacterium 1		9	1.7		
	β amylase limit dextrin 7		10	1.7		
	Leuconostoc dextranicum 1	> 324,000	9	2.2		
	Glycogen 2	> 550,000	10	2.6		
Mixed	Lemon pectin 8	> 20,000	20	1.6		
	Agar 9		25	2.2		
	Rhizobium trifolii 3		5	7.4		
Pentosan	Xylan 2	> 10,000	20	2.1		

* The sources of these substances were as follows: 1—Dr. J. P. Martin, Citrus Experiment Station, Riverside (described by Martin, 14); 2—Purchased from the Pfanstiehl Chemical Company; 3—Prepared in this laboratory (see text); 4—Dr. Dexter French, Iowa State College, Ames; 5—Dr. F. E. Brauns, Institute of Paper Chemistry, Appleton; 6—Dr. W. Z. Hassid, University of California, Berkeley; 7—Dr. W. J. Olson, Malting Laboratory, University of Wisconsin, Madison; 8—Purchased from Eastman Kodak Company; 9—Purchased from Difco Laboratories.
[b] Average value for the number of cuttings indicated.

the leaflet leaving turgid an area around the midvein. Under severe conditions (*e.g.*, by the use of a more concentrated solution), in both types the whole leaflet was often completely dry and necrotic. Such differences in symptoms did not appear related to the type of polysaccharide or linkage therein. Thus fructosans, glucosans, pentosans, and polysaccharides with mixed sugars all affected the leaflets.

The toxic unit value is defined as the product of sample dilution and toxic index divided by solution intake in milliliters (10). Thus a toxic unit is the amount of substance dissolved in 1 ml. of a solution that caused a toxic index of 1. In general the toxic unit is large when the solution intake is low.

However, some preparations (*e.g.*, black spruce polysaccharide) do not cause severe symptoms even though the solution intake is low.

The toxic unit value (Table 1) for glucosan from crown-gall bacteria is lower than previously reported (10). This probably is due to the use of somewhat larger cuttings in the present work.

The molecular weight of certain of these preparations was considered in relation to wilt-inducing ability. Unfortunately, it is difficult to obtain suitable samples of known molecular weights. Many polymers are not homogeneous but contain a mixture of molecular species that differ many fold in molecular weight. For many of the samples obtained the molecular weight is not known, and for others only an average molecular weight is known. Various reports[2] give molecular weight data on several of the types of substances tested. A molecular weight determination on each sample was beyond the scope of this work. Hence it is not known how closely the polymers tested correspond to those reported in the literature. However, the data in the upper part of table 1 suggest in general a direct relationship between molecular size and wilt-inducing activity on leaflets. As discussed later, the data suggest that the toxicity of these substances is due to a mechanical interference with the moisture relations of the plant.

Among the preparations causing stem and petiole wilting, the molecular weights of only a few are known.[3] As explained above no adequate means had been evolved for estimating the potency of these substances; hence a molecular weight-toxicity relationship was not readily demonstrated.

The amount of solution taken up by the cuttings presumably determines the actual weight of substance absorbed. Considerable variation was thus noted in the quantity of different substances taken into the plant during the 48-hour test period. This amount of substance is involved in the toxic unit value for those compounds having activity on leaflets. A smaller

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[2] Glucosan from *P. tumefaciens* has a molecular weight of about 3,600 (13). Harvey (4) lists inulin as 5,000 and soluble starch as about 4,000; however, our samples may have been different. The corn syrup dextrins were approximately 2,000, 1,200, and 1,000, respectively, for the 12 unit, 7.5 unit, and 5.4 unit molecules.

[3] The polysaccharide of *Betacoccus arabinosaceus* has a molecular weight of about 2,600 (5). It might be expected that this substance would cause wilting of leaflets. However, its low solubility in water may account for its wilting behavior on petioles and stems. Peat, Schlüchterer, and Stacey (17), from osmotic pressure measurements of a dextran produced by a strain of *Leuconostoc dextranicum (Betacoccus arabinosaceus haemolyticus)*, estimated a minimum chain length of 200 glucose units (molecular weight about 324,000). Methylated glycogen preparations from various sources were found by Carter and Record (1) to have chain lengths of 3,400 to 5,400 glucose units (molecular weights about 551,000 to 875,000) as measured by osmotic pressure. Oakley and Young (16), from osmotic pressure measurements, found rabbit liver glycogen to have a mean particle weight of the order of 2,000,000. Various pectin preparations may have molecular weights ranging from less than 30,000 to over 200,000, as indicated by Schneider and Fritschi (19) and by Speiser and Eddy (23). For example, the latter investigators fractionated a sample of nitrated pectin having an average molecular weight of 125,000. Molecular weight species ranging from 20,800 to 268,000 were obtained. The pectin tested in the present work induced slight wilting of leaflets and also of petioles. The leaflet symptoms may have been caused by the smaller molecules and the petiole symptoms by the larger ones. From viscosity measurements Haworth (7) estimated that xylan may have a chain length of 75 to 80 pentose units (molecular weight about 10,000 to 10,500).

quantity of substance having the higher toxic unit value was necessary to cause a similar toxic index.

Considerable variation also was noted in the amount of solution taken up by cuttings in solutions of substances causing wilting of petioles and stems. Thus about 4 mg. of polysaccharide of *Betacoccus arabinosaceus* and about 29 mg. of polysaccharide of *Rhizobium trifolii* apparently were absorbed per plant. In general less of the substances causing petiole and stem wilting was absorbed in the 48-hour test period than of those causing leaflet wilting.

Undoubtedly the shape of the molecule also is important. A molecule with short cross section would be expected to pass through a membrane perhaps more rapidly than one of large cross section when the pore size was limiting. However, the difficulty of obtaining suitable compounds prevented a study of this factor at present.

Activity of other polymers.—More data were sought on the suggested relationship between molecular weight and wilt-inducing activity. The polyethylene glycols were used since they are available in several molecular weight ranges. They are readily soluble in water and are reported to have low toxicity to plants (3, 15).

The polyethylene glycols have the general formula $HOCH_2$ $(CH_2OCH_2)_x$ CH_2OH where X varies with the molecular weight of the compound. Those with average molecular weights varying from 200 to 700 are liquids; those above 1000 are waxy solids called "Carbowax". For this work ethylene glycol, polyethylene glycols 200 and 400, and Carbowaxes 1540, 4000, 6000, and 9000 were employed. The number of each preparation corresponds roughly to the average molecular weight. However, the molecular weight of Carbowax 4000 has been estimated as 3,000 and 3,590, respectively, by independent methods (20). It is not known how closely the molecular weights of the other compounds actually conformed to their designated numbers.

Aqueous solutions (0.1 to 0.4 per cent) of several of these compounds were tested in the usual manner. Certain of them induced severe wilting of tomato cuttings. The symptoms were confined to the leaflets and were similar to those shown in figure 1, A and D. Ethylene glycol alone had mild activity. Polyethylene glycol 200 had somewhat greater activity but polyethylene glycol 400 had negligible activity. Carbowax 1540 induced a severe wilting of tips and edges of the leaflets; in some cases a pronounced uprolling of the edges and irregular, discolored, shiny areas appeared. The symptoms from Carbowax 4000 were like those from Carbowax 1540, but they were more severe. The symptoms from Carbowax 6000 were somewhat like the above. However, no necrotic areas and only a little leaf rolling appeared. Instead, the leaflet edges collapsed from the midvein and only a small area around the midvein remained turgid. Symptoms from Carbowax 9000 were similar to those of Carbowax 6000 but more severe. If the reported molecular weights of the various Carbowax compounds are

plotted against their wilt-inducing activities, in general the points fall along a straight line. This result was obtained in several trials. However, the slope of the line varied considerably from one trial to another. A representative experiment is shown in figure 2. The compound with the highest molecular weight is the most toxic. The behavior of the polyethylene glycols is thus similar to that of polysaccharides in that toxicity in general varies directly with molecular weight.
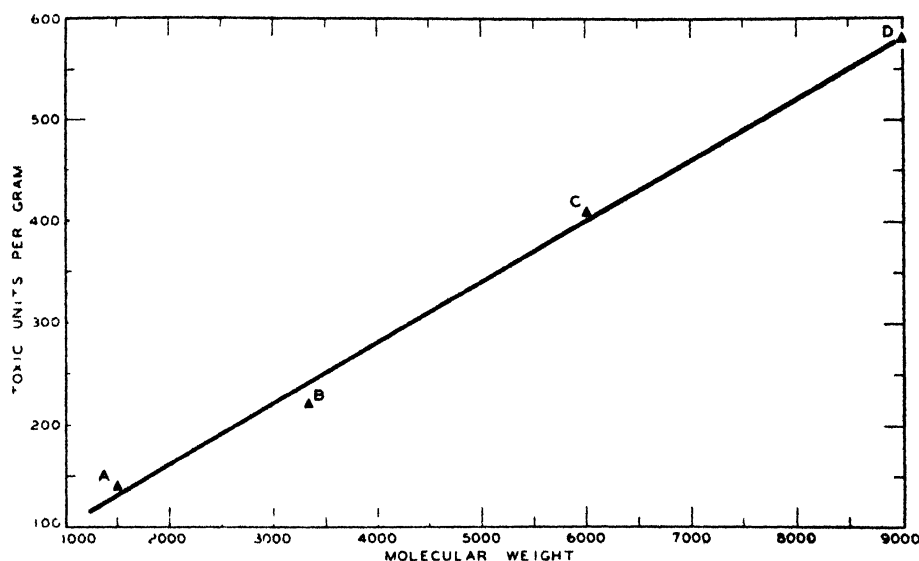
FIG. 2. Relationship of wilt inducing activity of Carbowax compounds (0.2 per cent solution) to molecular weight. Each point is the average of 5 cuttings. A. Carbowax 1540; B. Carbowax 4000; C. Carbowax 6000; and D. Carbowax 9000.

Another water-soluble polymer used was polyvinyl alcohol (Elvanol) which has been reported to be non-toxic to plants (24). Partially hydrolyzed types contain varying amounts of polyvinyl acetate in addition to polyvinyl alcohol; completely hydrolyzed types contain only polyvinyl alcohol. Both partially and completely hydrolyzed types are available in each of three viscosity ranges, i.e., low, medium, and high. For this work samples were obtained of the completely hydrolyzed type in each of the viscosity ranges. The approximate molecular weight ranges of these are 11,500, 40,000, and 52,000, respectively.

Aqueous solutions (0.2 per cent concentration) were tested on tomato cuttings in the usual manner. The low viscosity type was somewhat less toxic, and the medium viscosity type was somewhat more toxic than Carbowax 6000. Wilting induced by the low and medium viscosity types was confined mainly to the leaflets (similar to those shown in figure 1, A and D). The low viscosity sample induced severe rolling and wilting of tips and edges of the leaflets. That having medium viscosity caused severe wilting of leaflet edges with some rolling and leaflet collapse. That having

high viscosity differed from the above. Only slight leaf rolling appeared, but pronounced flagging of petioles and to a lesser extent the stems and leaflets was apparent (similar to symptoms in figure 1, B and C).

Again the relationship of molecular weight to wilt-inducing activity was evident. The potency of the medium viscosity type was considerably higher than that of the low viscosity type. Unfortunately the potency of the high viscosity type could not be estimated by this method since it induced a very different type of wilting. However, in general, with the two preparations a direct relationship appears between molecular weight and wilt-inducing power.

TABLE 2.—*Action of tomato leaf juice on soluble starch and glucosan from crown-gall bacteria*

Addition to plant juice[a]	Incubation time before heating[b]	Weight of polysaccharide added	Reducing sugar found[c]	Increase in sugar on incubation	Degree of hydrolysis
	Hr.	*Mg.*	*Mg.*	*Mg.*	*Per cent*
None	0	0	42		
Do.	72	0	46	4	
Soluble starch	0	40	48	.	
Do.	72	40	70	22	45
Glucosan from crown gall bacteria	0	40	45		
Do.	72	40	50	5	3

[a] The reaction mixture contained 10 ml. leaf juice, 10 ml. carbohydrate solution, and 2 ml. toluene.
[b] The heating time was 5 minutes at 100° C.
[c] Estimated as glucose by the micro-colorimetric method of Somogyi (22).

A few experiments were made with nitrogenous substances; *viz.*, filtered egg albumin, peptone, and yeast extract solutions. All these substances caused severe wilting of the leaflets of tomato cuttings (similar to those shown in figure 1, A and D). It is thus apparent that the wilting described is a nonspecific phenomenon which may be caused by a wide variety of substances.

Fate of the toxic substances in treated cuttings.—No entirely satisfactory explanation for the toxic effects of all the compounds tested has arisen. However, a few possibilities were explored.

It might be expected that a polysaccharide such as starch, which is a normal plant constituent and yet induces wilting, would be hydrolyzed readily by plant enzymes. However, a polysaccharide with β-glucosidic linkages, such as the glucosan from crown-gall bacteria, probably would not be readily attacked. These possibilities were examined.

Solutions of soluble starch and glucosan from crown-gall bacteria were incubated with crude plant juice expressed by pressure (6,000 to 12,000 lb. per sq. in.) from tomato leaves. After incubation for 72 hours at room temperature, reducing sugar was determined on each sample. The data (Table 2) indicated that about 45 per cent of the soluble starch, but only

3 per cent of the glucosan from crown-gall bacteria, was converted into reducing sugars. These data indicated a hydrolytic action of the plant juice upon starch. However, such action, if any, on the polysaccharide from crown-gall bacteria was slight.

The possibility was examined that hydrolysis of soluble starch might result in a toxic concentration of sugar. Leaves clipped from cuttings that had stood 48 hours in 1 per cent solutions of (a) soluble starch, (b) polysaccharide from crown-gall bacteria, (c) glucose, (d) sucrose, and (e) distilled water were dried at approximately 85° C. and ground to pass a 60-mesh sieve. Samples (about 0.5 gm.) were weighed into 50-ml. Erlenmeyer flasks, and 5 ml. of distilled water and 5 ml. of 1 N sulfuric acid were added. The mixtures were heated for 10 minutes at 100° C., were filtered, neutralized, and analyzed for reducing sugar.

TABLE 3.—*The sugar content of leaves from tomato cuttings in water and in carbohydrate solutions.*

Solution	Solution intake[a]	Toxic index[a]	Reducing sugar[b]
	Ml.		*Per cent*
Soluble starch	1.0	4.7	3.7
Crown gall polysaccharide	2 5	2.6	3.2
Glucose	5.6	0.2	6.2
Sucrose	6 2	0.0	7.4
Water	12.3	0.0	2.4

[a] Each value is the average of 5 cuttings.
[b] Sugar values were calculated as glucose on the dry weight basis.

The results (Table 3) indicated that sugar concentration was not the reason for the toxic activity of polysaccharides since sucrose- or glucose-treated plants, which showed no toxic symptoms, contained more sugar than the polysaccharide-treated plants, which had severe symptoms.

The distribution of polysaccharide from crown-gall bacteria was studied in treated cuttings. Hydrolysis (1 N hydrochloric acid at 100° C. for 2 hours) completely degrades this substance into glucose. However, very little glucose is formed from the substance in 10 minutes at 70° C., although sucrose is completely hydrolyzed in this time. Therefore, a rough measure of polysaccharide may be obtained by the difference in reducing sugar formed upon hydrolysis for 10 minutes and 2 hours. Of course, many other substances in the plant (*e.g.*, starch and hemicelluloses) would give rise to reducing sugar when subjected to such treatment. However, an approximate correction for such substances could be made by including appropriate controls.

Leaflets of tomato cuttings that had stood for 24 hours in various solutions were sectioned into wilted and non-wilted portions. These were dried, ground, hydrolyzed and filtered, and the filtrates were analyzed for reducing sugar. The results appear in table 4. The leaves of cuttings placed in

filtrate from crown-gall bacteria contained a greater quantity of poly-saccharide than those from cuttings in either water or in the regular sucrose, urea, mineral-salts medium used for culturing the crown-gall organism. With the bacterial filtrate a greater quantity of polysaccharide was found in wilted than in non-wilted portions of the leaflets. As might be expected, the wilted portions of the leaflets had a lower moisture content than the non-wilted portions.

TABLE 4.—*Analyses of normal and wilted tomato leaflets for polysaccharide* [a]

Material	Moisture	Dry weight	Polysaccharide
	Per cent	*Gm.*	*Per cent* [b]
Normal leaflets of cuttings in			
Tap water			
Centers	93	0.90	4.3
Edges	92	0.94	4.2
Unfermented medium			
Centers	92	1.15	3.4
Edges	90	1.19	3.2
Wilted leaflets of cuttings in			
Bacterial filtrate			
Centers	91	0.77	10.3
Edges	83	1.42	13.4

[a] Analyses were made on composite samples of 18 cuttings.
[b] Estimated as percentage of the dry weight.

Attempts were made to account quantitatively for soluble starch taken up by cuttings. Analyses for starch were made by the method of Hassid et al. (6) on treated cuttings sectioned into stems, petioles, wilted portions of leaflets, and non-wilted portions of leaflets. However, it was possible to account for only a portion (about 30 per cent) of the starch absorbed.

The distribution of the starch found varied with the particular preparation of soluble starch used. One sample was readily soluble in water, formed a clear solution and contained about 12 per cent of reducing sugar. Of that recovered, 49, 14, 9, and 27 per cent, respectively, were found in leaflet edges (wilted), leaflet centers (non-wilted), petioles, and stems. Another preparation of soluble starch was much less soluble in water, formed an opalescent solution, and contained no reducing sugar. Of the amount of this preparation recovered, 13, 16, 4, and 67 per cent, respectively, were found in leaflet edges (wilted), leaflet centers (not wilted), petioles, and stems. In this case most of the starch was in the stems whereas with another preparation most was in the leaflets. Possibly the particle size was too large to reach the leaf margins.

The low recoveries of absorbed starch probably were related to the low light intensity maintained in the test room. Under these conditions the cuttings probably were not synthesizing starch to any great extent, but were converting it into other substances.

A study was made of the distribution of polyethylene glycols in tomato cuttings that were treated with various glycol solutions. After 48 hours in

the solutions the cuttings were dissected into wilted and non-wilted parts of leaflets, petioles, and stems. Composites of several cuttings (usually 5) were dried at 75° to 90° C. in a stream of air, were ground to pass a 60-mesh sieve, and were analyzed for the respective glycols. Representative results are summarized in tables 5 and 6. The following items are particularly noteworthy.

While the toxic index remained somewhat similar, solution intake decreased rapidly with increasing molecular weight and, correspondingly, less of the large molecular-weight substance actually was absorbed by the cutting. Thus, about 18 mg. Carbowax 4000, but only about 6 mg. Carbowax 9000 were absorbed per plant.

TABLE 5.—*Analyses of tomato cuttings treated with Carbowax compounds*

Solutions	Solution intake[a]	Toxic index[a]	Carbowax absorbed[b]	Recovery of absorbed Carbowax[c]
	Ml.		*Mg.*	*Per cent*
Carbowax 1540[d]	11.7	3.2		
Carbowax 4000	9.2	4.0	92	97
Carbowax 6000	3.9	3.2	39	87
Carbowax 9000	2.9	3.3	29	94
Distilled water	24.6	0.0	0	

[a] Average value from 5 cuttings.
[b] Estimated from the total intake of 5 cuttings.
[c] Values corrected empirically for the "Carbowax" in control tissue.
[d] Composite of 4 cuttings, others composite of 5 cuttings.

Non-wilted portions of leaflets, petioles, and stems of all cuttings were very similar in moisture content. The moisture content of stems and petioles appears somewhat high. This probably is due to water soaking during the 48-hour test period, when the stems were standing in solutions from one to several inches deep.

The distribution of Carbowax in the various parts (calculated as a percentage of the total amount found in the cutting) is not correlated strictly with molecular weight of the particular Carbowax. However, if values for Carbowax 1540 and 4000 on one hand, and Carbowax 6000 and 9000 on the other, are considered, less glycol is present in the wilted portions of leaflets and more in the non-wilted portions with the larger molecular weight substances. This division also corresponds roughly to a subtle difference in symptoms induced, as previously mentioned, *i.e.*, Carbowax 1540 and 4000 have greater activity on leaflet edges than Carbowax 6000 and 9000.

An over-all recovery of 87 to 97 per cent of the glycol absorbed was obtained, and 81 to 91 per cent of the glycol was found in the leaflets. More Carbowax was found in wilted than in non-wilted portions of leaflets. In these cases also there is an accumulation of the wilt-inducing agent in the leaflets.

TABLE 6.—*Distribution of absorbed Carbowax in tomato cuttings*

Part analyzed	Moisture in wet tissue	Wt. dry tissue	Carbowax found[a]	Distribution of Carbowax[b]
	Per cent	Gm.	Per cent	Per cent
Carbowax 1540				
Leaflet edges (wilted)	72	0.63	10.8	68
Leaflet centers (non-wilted)	90	0.46	5.0	23
Petioles	96	0.22	0.7	1
Stems	97	0.48	1.7	8
Carbowax 4000				
Leaflet edges (wilted)	65	1.23	5.6	71
Leaflet centers (non-wilted)	89	0.51	3.2	17
Petioles	96	0.38	0.6	2
Stems	97	0.83	1.2	10
Carbowax 6000				
Leaflet edges (wilted)	81	0.89	2.5	54
Leaflet centers (non-wilted)	89	0.59	1.9	27
Petioles	96	0.33	0.8	7
Stems	97	0.66	0.7	12
Carbowax 9000				
Leaflet edges (wilted)	80	0.89	2.2	59
Leaflet centers (non-wilted)	89	0.57	1.9	31
Petioles	96	0.33	0.1	1
Stems	97	0.60	0.5	9
Distilled water[c]				
Leaflet edges (non-wilted)	90	0.79	0.2[d]	....
Leaflet centers (non-wilted)	92	0.71	0.3[d]	...
Petioles	96	0.34	0.2[e]	..
Stems	97	0.84	0.4[e]	..

[a] Calculated on the dry weight basis.
[b] Calculated as percentage of the total Carbowax found in the whole cuttings.
[c] A small amount of unknown substance in control tissue assays as Carbowax.
[d] Estimated as Carbowax 1540.
[e] Estimated as Carbowax 4000.

DISCUSSION

Among the polysaccharides and gums tested, two general types of wilting symptoms were observed. Some wilted principally the leaflets, others wilted principally the petioles and stems. In addition to polysaccharides, several other types of molecules, e.g., polyethylene glycols, polyvinyl alcohols, and nitrogenous substances, caused wilting primarily of the leaflets. Before any conclusions can be drawn as to the reason or reasons such a wide variety of substances caused similar wilting symptoms in leaflets, several relevant factors should be considered.

Moisture intake was related to the toxicity of a given substance. It should be emphasized that the wilt-inducing potency of a given preparation was estimated from the degree of wilting, the amount of solution absorbed by the cutting, and the concentration of the assay solution, as previously described (10). Thus an extremely potent wilt-inducing substance is one that at low concentration causes considerable wilting and allows only slight moisture intake. It appears that low moisture intake is either the result or the cause of wilting.

A structural consideration of several compounds tested, e.g., the polysac-

charide of crown-gall bacteria, indicates no especially reactive functional groups, such as carboxyls, that might be expected to impart toxicity to the molecule. If toxicity could be assigned to such a functional group, the potency of the compound would be expected to be directly related to its molar concentration. However, among the members of each group of the wilt-inducing substances tested, when the molecular weight was reasonably clear, the largest molecules were also the most toxic. Since all substances were prepared for assay on a weight basis rather than on a molar basis, solutions of the large molecular weight substances contained fewer molecules per unit volume than those of low molecular weight. Yet the solution containing the fewer molecules had greater wilting potency. The compounds having large molecular weights thus appear more toxic simply because they are larger in physical dimensions.

The best explanation as yet available from the evidence is that the various compounds tested induce wilting because they mechanically interfere with the transpiration system of the plant. The exact locus or loci of such an interference are not clear and cannot be assigned on the basis of present information. However, the analytical data indicate that many of the substances, e.g., Carbowax compounds, pass through the large vessels of the stem and petiole and accumulate mainly in the leaflets, particularly in the margins. A mechanical blocking of the transpiration stream possibly at the ends of the tiny veinlets might account for the greater toxicity of the larger molecules. Because of their physical size, the large molecules might be more effective than smaller ones in blocking movement of solutions through a membrane.

Furthermore, the possibility remains that some of these substances actually might diffuse through the cell membrane or pass through the plasmodesma and accumulate within the cells. In such cases they perhaps might interfere with the activity of cell contents.

That soluble starch, a normal plant constituent, should cause wilting even though amylases are present in the leaves, deserves comment. Probably the plant amylases are located principally within the cells rather than in the vessels. Then in treated cuttings such enzymes would have little contact with the starch until it entered the cells. Passage of a moderately large molecule through the cell membrane at best probably would be slow. Thus it appears that large molecules that are sufficiently soluble to be transported in the plant sap and that are too large to move rapidly into the cells might pile up in the ends of the tiny veinlets.

Polysaccharide of crown-gall bacteria in aqueous solution diffused slowly through a cellophane membrane. Since it apparently is not hydrolyzed to any appreciable extent by tomato leaf juice, it might also induce wilting even if it passed readily from the vessels into the cells and accumulated there rather than in the ends of the tiny veinlets.

The present work indicates that the Carbowax compounds are toxic in low concentration when introduced into the vascular system of tomato cuttings.

Concentrations of 0.1 per cent of several Carbowax compounds induced marked symptoms within 48 hours. However, as surface applications these compounds are less toxic. Mitchell and Hamner (15) tested various concentrations of Carbowax in aqueous solution on several plants. Tomato plants were not injured with 1 per cent solutions. Withrow and Howlett (24) reported that undiluted Carbowax 1500 and Carbowax 4000 were very toxic to tomato leaf tissues and flowers. However, none of the leaf tissues of several plants were injured with 1 per cent or less of Carbowax in aqueous solution. On the other hand, tomato floral parts were injured by as little as 0.1 per cent Carbowax.

Certain compounds of the types studied, or related to these, are produced by plant pathogens and have been shown to induce wilting in plants. The polysaccharide of the crown-gall organism induces a wilting and necrosis of tomato leaflets (10). The organism causing the Dutch elm disease has been shown by Dimond (2) to produce (a) a substance that appears to be a polysaccharide and that causes curling of leaflet edges and (b) an unidentified substance causing severe necrosis. Plattner and Clauson-Kaas (18) isolated from a fusarium culture, a peptide which induced wilting similar in appearance to that caused by the glucosan from crown-gall bacteria.

Apparently many organisms produce substances of moderate molecular size. The possibility deserves further study that such metabolites may have important effects largely through mechanical interference in the large vessels, in the small vein islets, or inside the cells.

SUMMARY

The wilt-inducing properties of various groups of polymers (in 0.1 to 0.4 per cent aqueous solution) upon tomato cuttings have been studied under controlled conditions of light, temperature, and relative humidity.

Various polysaccharides of both plant and microbial origin induced wilting in tomato cuttings. Those that were water-soluble and formed clear solutions induced wilting of the leaflets. One of these was shown to accumulate in the leaflets and especially in their margins. Those less soluble and forming opalescent solutions usually caused flaccidity, particularly of petioles and stems. Certain of these apparently caused plugging at the base of the stem. Among those that caused leaflet wilting a direct relationship, in general, appeared between molecular weight and wilt-inducing ability.

Among the polyethylene glycols, Carbowaxes 1540, 4000, 6000, and 9000 were studied particularly. These all caused wilting of the leaflets. A direct correlation again appeared between wilt-inducing potency and molecular weight. Chemical analyses indicated that 81 to 91 per cent of the compound absorbed accumulated in the leaflets, with the highest concentration appearing in the wilted portion.

Among the polyvinyl alcohols, those of low and medium molecular weights, e.g.. 11,500 and 40,000, induced wilting primarily of leaflets. A

third compound (molecular weight about 52,000) caused flaccidity, particularly of petioles and stems. A direct relationship between molecular weight and toxicity again appeared with those preparations causing leaflet wilting.

The data obtained indicated that the wilting induced by these polymers may be correlated with molecular weight. Toxicity could not be assigned to any functional group common to such a diverse group of substances. The data seemed best explained by assuming that these substances were toxic because they mechanically interfered with the transpiration system. While the loci of such an interference could not be assigned on the basis of present evidence, it seemed possible that these substances might accumulate, for example, in the large vessels at the ends of the vein islets, or inside the cells, depending in large part on their size.

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SELECTION AND PROPAGATION OF ALBIZZIA FOR RESISTANCE TO FUSARIUM WILT

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The mimosa tree (*Albizzia julibrissin* Durazz.) is used extensively as an ornamental in southern United States, where it is valued for its rapid growth, graceful foliage, and colorful flowers. A destructive vascular wilt disease, caused by *Fusarium oxysporum* f. *perniciosum* (Hepting) Toole (1, 4, 6), presents a threat to the future of this tree in the country.

The selection and propagation of lines of the mimosa tree resistant to the wilt offered a possible solution to the problem of control. The control of Fusarium wilts through soil treatments and amendments has not proved generally practicable, and even if successful, such treatments may be expensive or a nuisance when applied over the life of a tree. Results are reported herein of experiments carried on since 1939, to discover and propagate wilt-resistant mimosas. Some of the early efforts along these lines have already been briefly reported (2, 3).

TESTING OF SEEDLINGS FOR WILT-RESISTANCE

Although careful watch had been kept for mimosa trees resistant to or immune from the Fusarium wilt in some of the heavily wilt-infested areas, no such trees were found. Therefore, tests were made of the susceptibility to wilt of seedlings from a large number of trees chosen at random over much of the range of the mimosa in the southern states. Seed was obtained in the fall of 1939, from 50 trees of *Albizzia julibrissin* scattered from Maryland to Louisiana, 1 tree of *A. julibrissin* var. *rosea* (Carr.) Mouillef. from Jamaica Plains, Mass., and 2 trees of *A. kalkora* (Roxb.) Prain., 1 each from Savannah, Ga., and Biltmore, N. C.

In the first experiment (Table 1) seedlings from these seed lots, grown in flats in the greenhouse during the winter of 1939-40, were tested for resistance in the spring of 1940 by inoculation in the greenhouse, with highly virulent strains of the wilt *Fusarium*, by procedures previously described (6). All the seedlings from most parent seed lots of *Albizzia julibrissin* died of wilt by the end of the 1940 growing season. However, some seedlings in a few of these groups, together with several seedlings of *A. julibrissin* var. *rosea* and *A. kalkora*, remained healthy. These survivals were given a second inoculation in the greenhouse in the spring of 1941, and the 30 seedlings still alive the following season were inoculated a third time and planted in naturally wilt-infested soil near Tryon, N. C.

In a second experiment (Table 1) additional seedlings were grown from the seed of the 4 parent trees that yielded the most resistant progeny in 1940. These, together with seedlings from a susceptible tree, were inoculated in the spring of 1941. The 64 surviving seedlings were inoculated a second time in 1942 and planted in naturally wilt-infested soil.

Many of the trees set out at Tryon in 1942 died within a month or two after they were transplanted, because of the extremely dry season, but they

TABLE 1.—*Summary of inoculations on selections of mimosa seedlings*

Experiment[a]	Species of Albizzia	Parent seed lots	Original seedlings inoculated	Trees alive in 1948
Number		*Number*	*Number*	*Number*
1	*A. julibrissin*	2	15	5
	A. julibrissin	48	444	0
	A. julibrissin var. *rosea*	1	9	1
	A. kalkora	2	20	2
2	*A. julibrissin*	2	64	12
	A. julibrissin	3	80	0
3	*A. julibrissin*	10	336	203
	A. julibrissin	9	317	0
	A. julibrissin var. *rosea*	2	7	6
	A. kalkora	2	47	43
4	*A. julibrissin*	4	98	55

[a] The seedlings in experiment 1 were inoculated in 1940, 1941, and 1942; those in experiment 2 were inoculated in 1941 and 1942, and those in experiments 3 and 4 were inoculated in 1947.

had no symptoms of Fusarium wilt. A few others were accidentally mowed down. Twenty trees from these first two experiments, representing 4 different parent trees of *Albizzia julibrissin* and 1 each of *A. julibrissin* var. *rosea* and *A. kalkora* have remained alive through six growing seasons in the field (Table 1), while neighboring volunteers have died of the wilt disease. These 20 trees unquestionably possess some degree of resistance to mimosa wilt.

In experiment 3 (Table 1), started in 1946, the material grown and tested for resistance comprised seedlings from certain lots of 1939 seed that yielded resistant trees in experiments 1 and 2; seedlings from 1946 seed from these same trees; and seedlings from 1946 seed from a number of untested trees. They were inoculated in the spring of 1947, and at the end of the first season important differences in resistance were apparent (Table 1). The parent trees which yielded resistant progeny in earlier tests, using the original 1939 seed, again gave a high percentage of resistant seedlings; also, new collections from these trees gave additional resistant seedlings; and additional resistant parent trees were discovered from the 1947 tests.

In 1946, several of the 6- and 7-year-old resistant trees discovered in the first trials yielded seed for the first time. These seeds were collected

and seedlings from them were tested by inoculation in the greenhouse in 1947 (Experiment 4, Table 1). Fifty-six per cent of these seedlings survived one inoculation, compared with no survivals among seedlings from randomly selected trees. With uncontrolled pollination, it is obvious that progeny from resistant trees cannot be depended upon for resistance.

<div align="center">VEGETATIVE PROPAGATION</div>

Because wilt-resistant trees do not pass on the factor for resistance to all of their seedling progeny, it becomes necessary to propagate these trees vegetatively to increase the quantity of resistant stock. The remainder of this paper reports the results of methods of propagation of the mimosa by means of cuttings and testing them by inoculation.

Hardwood Stem Cuttings

Experiments were started to determine ways of rooting mimosa cuttings, without regard to wilt resistance. The resistant trees could not provide adequate cutting material and so hardwood stem cuttings were taken from wildling mimosa trees. A group of young vigorous trees growing near Asheville, N. C., was selected for stock material, and on March 24, 1945, shoots several feet long were cut from these trees and placed in water. Soon after collection the shoots were cut into sticks approximately 8 inches long and a record was made of their basal diameters. Twelve cuttings were available for each of 6 treatments. These treatments included dipping the lower ends in: (1) tap water for 20 hours; (2 and 3) indolebutyric acid at 40 p.p.m. and 500 p.p.m., respectively, for 20 hours; (4 and 5) naphthaleneacetic acid at 40 p.p.m. and 500 p.p.m., respectively, for 20 hours; and (6) 2-4-D at 50 p.p.m. for 1 hour. The upper ends of 6 cuttings in each treatment were dipped in wax, while the other 6 were not waxed. At the end of the treatment period, all cuttings were rinsed in tap water and planted in moist sand in flats in the greenhouse.

Four months after planting, all cuttings were dug and examined. Those that had not rooted were dead. Rooting had occurred only in cuttings that were treated with indolebutyric acid at 40 p.p.m., naphthaleneacetic acid at 40 p.p.m., or 2-4-D at 50 p.p.m. With one exception, cuttings less than 0.3 inch in diameter had not rooted. Rooting of larger cuttings was aided by waxing the tops: All the larger, top-waxed cuttings treated with indolebutyric acid at 40 p.p.m. rooted, whereas only 50 per cent of similar unwaxed cuttings rooted. Cuttings treated with indolebutyric acid are in figure 1.

In the spring of 1946, another experiment was undertaken, to compare the rooting of stem cuttings from resistant versus random wildling trees, and to provide a source of cuttings for inoculation tests comparing cuttings from these sources. Shoots were used from 6 of the resistant mimosa trees at Tryon, N. C., 3 nearby wildling trees, and 8 wildling trees from

Asheville, N. C. Two vigorous shoots from each tree were cut on March 11, 1946, and placed in water. The next day they were cut into 8-inch lengths, the upper ends waxed, and the bases set in a solution containing 40 p.p.m. of indolebutyric acid. After 24 hours, these cuttings were rinsed and planted in sand flats in the greenhouse. They were dug one year later. Four per cent of the cuttings from one resistant tree had rooted, 50 per

FIG. 1. Hardwood mimosa cuttings one fifth natural size, 4 months after treatment [with indolebutyric acid, 40 p.p m. for 20 hours] The two unrooted cuttings on the left are under 0.3 inch in diameter, and are dead.

cent from another, and none from the remaining 4. The Asheville wildling cuttings and cuttings from 2 of the 3 Tryon wildling trees gave 75 per cent rooting. Cuttings of the third Tryon wildling, which was low in vigor because of wilt infection, failed to root.

To test the value of several growth regulators at different concentrations and under various treatment conditions, the following experiment was set up, with shoots from vigorous trees growing near Asheville, N. C. Cuttings were made in January, 1946, and stored outdoors in moist sand for one month. At this time the cuttings were divided into sets of 6 and, after waxing the upper ends, treated as follows: (1) 100 p.p.m. indoleacetic acid for 24 hours; (2) 40 p.p.m. indolebutyric acid for 24 hours; (3) 100 p.p.m. indolebutyric acid for 24 hours; and (4) no treatment. One-half of each set was dipped in Fermate before planting in flats of vermiculite in the greenhouse. Within one month, 17 per cent of those cuttings given indolebutyric acid at a rate of 40 p.p.m. and 67 per cent of those treated with this acid at a rate of 100 p.p.m. had rooted. All other cuttings had died. There was no apparent rooting benefit from the Fermate treatment. This experiment gives further support to the previous

conclusions regarding the effectiveness of indolebutyric acid, and indicates that a concentration of 100 p.p.m. is much better than 40 p.p.m.

Another experiment was undertaken to test the value of various storage periods and collection times before treatment and planting of cuttings. Stem cuttings were collected in November, 1946, and in January and March, 1947, from 4 resistant trees at Tryon, N. C., a nearby wildling tree, and a wildling from Asheville. These cuttings were stored outdoors in moist sand until April 30, 1947, when they were dug up, soaked for 24 hours in a solution of indolebutyric acid 40 p.p.m., and planted in flats of vermiculite in the greenhouse. At this time the advantage of the 100 p.p.m. concentration had not been determined. Two and one half months after planting, all cuttings stored for 155 days and for 106 days had died. Many of those stored for 51 days were alive but unrooted. At this time they were transferred to a special propagation box similar to that described by Stoutemyer et al. (5), and in 2 weeks the cuttings from 3 of the 4 resistant trees had rooted as follows: 59 per cent, 100 per cent, and 24 per cent; while 57 per cent and 13 per cent of the cuttings from the 2 wildlings had rooted. Cuttings not rooted died within one month. This experiment, together with those already described, shows that individual mimosa trees differ with respect to the rootability of hardwood cuttings.

Greenwood Stem Cuttings

A number of attempts were made to root greenwood cuttings clipped from random field trees in 1945 and 1947, and placed in the propagation box after treatment with hormones. All such cuttings died within 2 weeks without rooting. In another experiment, where rooting of succulent sprouts from root and stem pieces was compared, it was found that only those greenwood cuttings that arose as sprouts from root pieces rooted (7). Much of the variability in rooting from large and small trees and from different parts of the same tree, experienced by several investigators, may be explained on the basis of the nearness of the cutting to the root system.

Root Cuttings

Mimosa has been observed to send up sprouts from cut roots and it seemed possible that propagation from root cuttings would be successful. Roots were collected from 4 resistant trees at Tryon, N. C., a nearby wildling, and a wildling mimosa near Asheville, N. C., in November, 1946, and in January and March, 1947. The roots were divided into cuttings 3 inches long and stored in moist sand outdoors until April 30, 1947, when they were treated with indolebutyric acid at 40 p.p.m. for 24 hours, and planted vertically in the greenhouse in flats of vermiculite, so that about ¼ inch of the top end was above the medium. Three months after planting, pieces not rooted were dead. There was no advantage in the longer storage periods, and in some cases they proved detrimental if cuttings became molded during storage. There were great differences in the readiness

with which cuttings rooted from different parent trees. Cuttings from the 4 resistant trees rooted 100 per cent, 50 per cent, 56 per cent, and 89 per cent, respectively, while those from the 2 wildling trees rooted 50 per cent and 42 per cent, respectively.

A comparison was made of the rooting of cuttings in a heated and artificially lighted propagation box and in the greenhouse. Root cuttings were collected on June 11, 1947, from 2 resistant trees and a nearby wildling

TABLE 2.—*Rooting of root cuttings of Albizzia julibrissin after one month in a propagation box and in the greenhouse*

Source	Propagation box		Greenhouse	
	Cuttings rooted		Cuttings rooted	
	Number	Per cent	Number	Per cent
Tryon wildling tree	30	80	29	35
Resistant tree No. 617ᵃ	22	0	21	0
Resistant tree No. 65	16	56	17	6

ᵃ This tree is of low vigor.

mimosa at Tryon; after treatment with indolebutyric acid at 40 p.p.m. for 24 hours, they were divided into two lots. One lot was planted in the propagation box and the other in the greenhouse in vermiculite. After one month a much higher percentage of those in the propagation box had rooted than of those in the greenhouse (Table 2). However, after 3 months, more of those cuttings in the greenhouse had rooted, so that the final percentage rooted under the two conditions was nearly the same. The propagation box had the advantage of hastening the rooting of root cuttings.

Inoculation of Cuttings

As a further check on the resistance to wilt of the original resistant selections, rooted stem cuttings from resistant trees and wildling trees were inoculated in April, 1947 with highly virulent strains of the mimosa wilt

TABLE 3.—*Resistance of rooted mimosa cuttings to Fusarium wilt*

Description of cutting	Cuttings inoculated April, 1947		Cuttings inoculated October, 1947	
	Original	Alive April, 1948	Original	Alive April, 1948
	Number	Number	Number	Number
Stem cuttings				
From Tryon resistant trees	8	8	8	8
From Tryon wildling trees	17	0	7	0
From Asheville wildling trees	23	2	8	0
Root cuttings				
From Tryon resistant trees			86	86
From Tryon wildling trees			22	0
From Asheville wildling trees			8	0

fungus, *Fusarium oxysporum* f. *perniciosum*, by methods previously described (6). After 1 year, all of the cuttings from resistant trees were alive, whereas none of the cuttings from wildling mimosas from Tryon survived the wilt (Table 3). Inoculations of additional root and stem cuttings in October, 1947, again indicated that cuttings from apparently resistant trees are resistant to the wilt disease (Table 3).

DISCUSSION

Certain individual mimosa trees are resistant to or immune from the common virulent strains of the mimosa wilt *Fusarium* used in the tests. Although seed from uncontrolled pollination of these trees produced trees, some of which were not resistant, all cuttings rooted from the resistant trees were wilt-resistant. This gives hope that the wilt disease can be alleviated through the replacement of susceptible individuals with resistant clones. However, because of the physiological and morphological variability that has been demonstrated for *F. oxysporum* f. *perniciosum* (4, 6), it is possible that a new form of the fungus may arise in nature, which will attack the present resistant clones.

The mimosa wilt is spreading rapidly, and although many of the resistant trees discovered so far are located outside the known range of the disease, other collections from these outlying areas are susceptible to the disease.

Resistant selections of *Albizzia julibrissin* var. *rosea* and *A. kalkora* may be valuable substitutes for *A. julibrissin*. Other species of *Albizzia* tested, which have some wilt resistance, are tropical or subtropical and not hardy within much of the area where the mimosa is valued.

SUMMARY

In testing certain species of *Albizzia*, mainly *A. julibrissin*, for resistance to Fusarium wilt, 1437 seedlings have been grown from seed collected at various locations from Maryland to Louisiana, and their roots inoculated. Twenty of these trees have survived the disease as long as 8 years, despite repeated inoculations, and in the more recent experiments many more have survived for shorter periods. Fifty-six per cent of the seedlings grown from seed resulting from uncontrolled pollination of the resistant selections were wilt-resistant.

All cuttings rooted from the resistant selections have thus far appeared to be immune from the wilt despite successive inoculations, while rooted cuttings from neighboring nonresistant wildlings became diseased and died following inoculation.

Experiments demonstrated the rooting superiority of root cuttings over stem cuttings, hardwood over greenwood cuttings, the use of the propagation box rather than the greenhouse bench, and no storage rather than prestorage of cuttings in moist sand.

Root cuttings rooted well when planted without hormone treatment; hardwood cuttings rooted well if they were from vigorous young trees, were thicker than 0.3 inch, were waxed at the upper end, and soaked in a suitable hormone solution; greenwood cuttings rooted well and without hormone treatment only if they had sprouted from root cuttings.

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ARSENITE SPRAY INJURY TO GRAPE CANES
THROUGH LEAF SCARS

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The application of sodium arsenite to grape vines (*Vitis vinifera* L.), during the dormant season to control black measles is an established practice in the vineyards of California. Spraying the trunk and arms of the Thompson Seedless variety during the late dormant season with sodium arsenite will result in approximately 80 per cent control of this disease.

Periodically there have been reports, particularly from some of the growers in the San Joaquin Valley, that bud injury resulted from this practice. Such injury was especially severe in 1946 and 1947, when an abnormally large number of buds on arsenite sprayed vines failed to produce new growth in the spring. Necrosis of varying degrees was associated with these abortive buds, particularly just below the subtending leaf scar.

Similar symptoms were observed on peach twigs by Haenseler and Martin (2) and also by Poole (3). All concluded the injury was due to the toxic effect of the arsenical content of the spray. Adams (1) made similar observations on peach twigs and arrived at the same conclusion as to the cause of the necrosis. He also noticed, a correlation between severity of premature defoliation and the number of twig lesions that appeared later. On the basis of this observation Adams postulated that the toxic materials of the sprays may gain entrance into the twigs through unhealed leaf scars, or even by way of the leaf traces. Wilson (4) noted that spraying apricot and almond with a dormant spray of monocalcium arsenite in January instead of December markedly reduced bud killing. He concluded that bud killing was due to soluble arsenite gaining entrance into the buds through the unhealed leaf scars.

BUD FAILURE CAUSED BY ARSENITE SPRAY

Spray plots were established during the fall of 1947 in three different Thompson Seedless vineyards in Fresno and Tulare Counties. The soil of two of the vineyards was dry in December and received no water from irrigation or rainfall until February. The soil of the third plot, designated wet was irrigated the first week of December.

The vines were sprayed with a sodium arsenite solution, containing an equivalent of 2¼ lb. arsenic trioxide per 100 gallons of water. Three blocks of vines consisting of 2 rows of 26 or more vines each were sprayed in each of the 3 vineyards on December 15. Additional blocks of vines in the same vineyard were sprayed January 20 and others on February 16. The spray

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was applied to the vines of two of the blocks in each vineyard in such a manner that only the trunks and arms were sprayed. In the third block the canes as well as the arms and trunks were sprayed.

To determine the amount of damage due to the arsenical spray, bud counts in groups of 100 were made on May 7. All of the buds on each individual cane selected at random in treated and nontreated rows of vines were counted. The number of buds which failed to produce any new growth were noted and expressed directly as percentage bud failure. Table 1 gives a summary of these counts for the different spray treatments on wet soil and dry soil plots.

TABLE 1.—*The average percentage of bud failure on sprayed and nonsprayed Thompson Seedless vines (May 7, 1948)*

Plot	Month sprayed	Sprayed trunks and arms only	Nonsprayed	Sprayed trunks, arms and canes	Nonsprayed
A (dry[a])	Dec.	18.2		32.5	
	Jan.	19.7	15.0	23.0	13.0
	Feb.	22.8		34.0	
B (dry[a])	Dec.	22.2		17.5	
	Jan.	22.5	17.5	30.5	17.0
	Feb.	21.7		27.5	
C (wet[a])	Dec.	22.0		22.5	
	Jan.	21.5	17.0	19.7	18.5
	Feb.	18.5		24.5	

[a] Dry—No fall irrigation.
  Wet—Fall irrigation.

Whether the vines were sprayed in December, January, or February there appeared to be little, if any, consistent difference in the amount of bud failure.

The slightly higher percentage of bud failure in the rows where the spray was applied only to the trunks and arms over the corresponding checks was probably due to injury caused by arsenite spray accidentally applied to the lower ends of the canes. Bud failure in these rows was most evident at the base of each cane.

On dry soil plots in which the canes were also sprayed the percentage of bud failure in treated rows was considerably greater than in the checks. On the wet soil plot, whether the canes were sprayed or not made no apparent difference in the amount of bud failure, even though in January an examination of leaf scars of canes sprayed in December showed that the traces were necrotic at the point of emergence from the vascular cylinder. However, the necrotic condition did not appear to extend below this point nor to involve the bud immediately above the scar. On the other hand, examination of sprayed buds from the dry soil plots showed necrosis had extended considerably farther below the leaf scar in the vascular cylinder as well as upward to the bud.

HISTOLOGICAL STUDIES OF HEALTHY AND INJURED TISSUES

Nodal segments were collected from the canes of nonsprayed vines on each of the spray dates, and again on May 7. On January 20, February 16, and again on May 7 nodes were collected from the canes of vines previously sprayed. As the nodes from the canes were collected they were stored in Formalin-acetic-alcohol killing and fixing solution. Material for study was sectioned with a sliding microtome, stained in Delafield's Hematoxylin, and mounted in balsam, following standard schedules.

A histological study of the nodes from nonsprayed vines showed that the periderm over the leaf scar was formed prior to leaf fall (Fig. 1, A), and


FIG. 1. Photomicrographs of radial sections through median leaf traces of non-sprayed nodes (×10). A. Node collected December 15. Periderm covering the leaf scar above the trace is well developed, (indicated by arrow). The trace is relatively free of gum and the broken ends of the vessels are exposed to the atmosphere. B. Node collected January 20. Wound gum (stained dark) has plugged some of the vessels. Others, however, are still open. C. Node collected February 16. Wound gum has completely plugged all of the vessels of the trace.

that the vessels of the leaf traces were open, and became plugged with wound gum much later. Fig. 1, A, from a radial section of a node collected in December, shows the vessels of the median trace to be relatively free of gum. This condition was apparent in approximately 90 per cent of such nodes examined. In the remaining 10 per cent, many of the vessels were plugged with gum, but in no case were all the vessels of a trace completely plugged. The terminal end of the trace was mechanically broken at leaf fall and the open vessels fully exposed to the atmosphere (Fig. 1, A). A similar section from a node collected in January (Fig. 1, B) shows considerable gum formation in some of the vessels of the trace, though several were still open. Approximately 70 per cent of such nodes examined, showed varying degrees of gum plugging in the traces; about 10 per cent were free
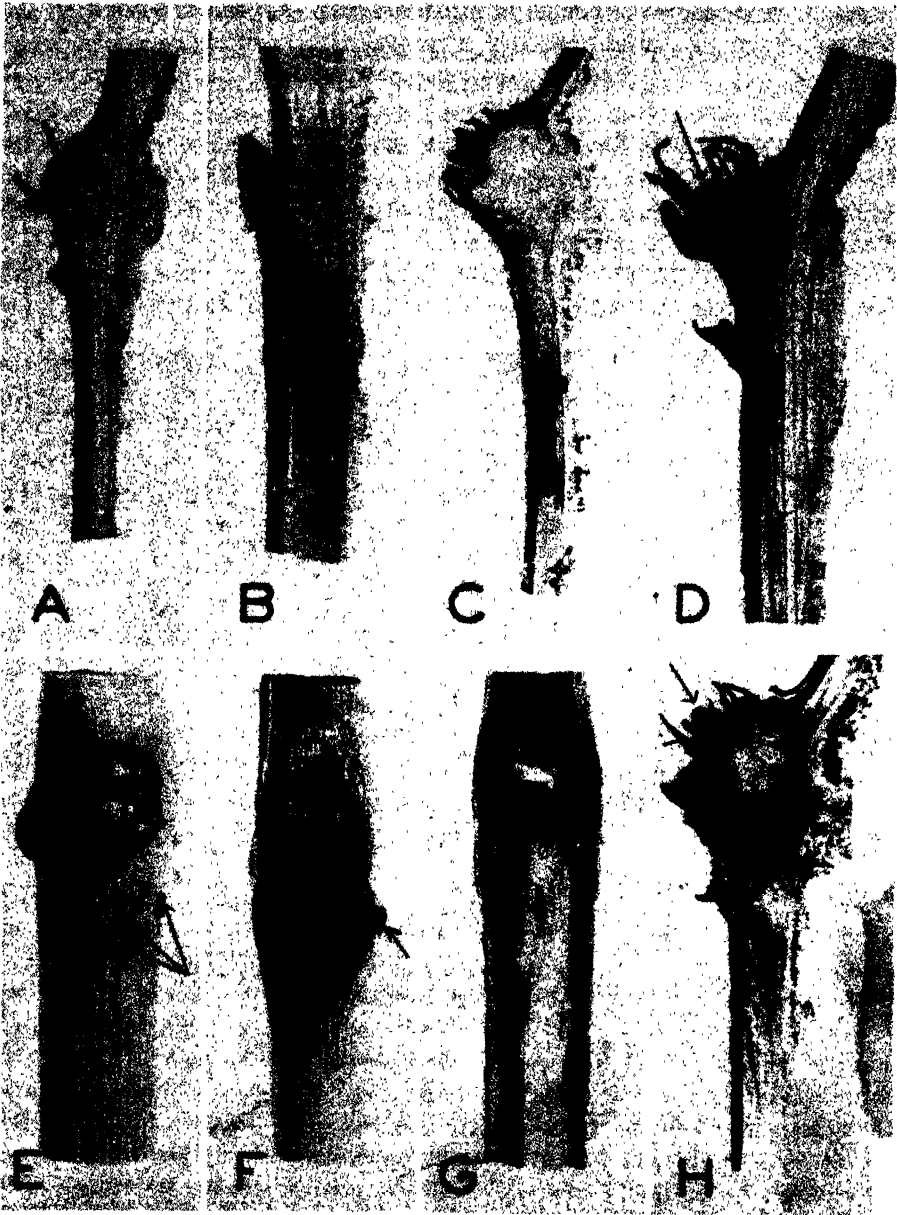
FIG. 2. A, B, C, D and H. Photomicrographs of median radial sections from nodal segments, of Thompson Seedless grape canes. E, F, and G. Photographs of nodal segments with bark removed. A. Section from a nonsprayed node with only the terminal portion of the median trace included (×3). B, C, D and H. Sections from previously sprayed nodes. B. Necrosis (stained dark) has spread downward along the trace and laterally in the medullary ray (×5). C. More advanced stage of necrosis in the xylem tissue (×3). D and H. Upward spread of the necrosis from the leaf trace has killed the bud (×1.5). E. Nonsprayed node with arrows indicating broken ends of leaf traces (×2). F. Necrotic sprayed node. Small necrotic area (indicated by arrow) showing the location of a damaged leaf trace and also showing necrosis below the bud following intake of toxic spray materials through the leaf traces (×2). G. Necrosis of the vascular cylinder which extended to a distance of 4 inches below the bud (×2).

of gum and about 20 per cent were completely plugged. A section from a node collected in February (Fig. 1, C) shows all of the vessels of the trace completely plugged with gum. All such nodes examined were in this condition.

Nodes from vines sprayed with sodium arsenite were necrotic where the trace emerged from the vascular cylinder (Fig. 2, F) of the cane and downward, especially along the trace, in some cases as far as 4 inches below the leaf scar (Fig. 2, B, G). From some traces necrosis also extended laterally into the xylem along some of the medullary rays (Fig. 2, C) and upward into the bud (Fig. 2, D, H).

Since necrosis was found only in the tissues of the nodes from sprayed vines, it was assumed that the injury was due to the entrance of the sodium arsenite into the cane tissues. It appeared from the sequence of nodes examined that the arsenite spray entered the canes through the open and exposed ends of the broken leaf traces, moved downward through the vessels of the trace and radially through some of the medullary rays. Upward movement may have been by diffusion from excessive intake through the leaf trace or by way of the vascular tissue from radial movement in the rays.

SUMMARY

Sodium arsenite applied as a dormant spray to the canes, arms, and trunks of Thompson Seedless vines, (*Vitis vinifera* L.), on dry soil plots, caused appreciable injury as evidenced by sharply defined necrotic areas in the xylem tissue below the leaf scar and bud killing. Vines sprayed only on the arms and trunks had considerably less injury, and it was confined chiefly to lower nodes of the canes. Sprayed vines in a wet soil plot showed only minor xylem injury with little bud killing.

Histological study showed that the leaf scar periderm was formed before leaf fall. Most of the vessels of the leaf traces in scars collected in December after leaf fall were open with no covering over the broken ends and little to no wound gum within the vessels. Some of the vessels in approximately 70 per cent of the leaf traces collected in January were plugged, all of the vessels in about 20 per cent were plugged, and only about 10 per cent showed little or no plugging of the vessels with gum. By February, plugging of the vessels of the leaf scar traces was complete.

A study of damaged buds indicated that the sodium arsenite solution penetrated to the xylem tissue through the vessels of the leaf trace, that necrosis extended downward as far as 4 inches below the leaf scar and also upward into the bud.

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DIFFERENCES IN PYTHIUM INJURY TO CORN SEEDLINGS AT HIGH AND LOW SOIL TEMPERATURES

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A type of injury from *Pythium* sp. to germinating seed corn, different from that which commonly occurs in cold, wet soils (root rot and seed decay), was observed in a recent greenhouse experiment conducted in ordinary compost soil at a temperature range of 20°–24° C. which is very favorable for germination and seedling growth. Seedlings from nearly disease-free kernels that had been wounded by removing a portion of the crown of each, thus exposing the starchy endosperm, were considerably stunted when compared with those from seed similarly injured but which had been dusted with Arasan seed protectant (tetramethyl thiuram disulphide).

Except for their reduced size, the stunted seedlings had few symptoms ordinarily caused by the common seedling blight fungi. Mesocotyl tissues generally were sound and the root systems appeared normal for seedlings of their size. If it had not been for the comparison afforded by the seedlings from the treated seed, the sub-normal conditions of the stunted seedlings (Fig. 1) might have been unnoticed.

Evidence that micro-organisms were involved in the stunting was indicated in the condition of the nontreated kernels still attached to the seedlings. The wounds at the crowns were covered with agglutinized soil and the endosperm and scutellum tissues were more decomposed than in the kernels that had been treated with Arasan. These signs of fungus invasion suggested platings for the isolation of pathogens.

ISOLATIONS AND ARTIFICIAL INOCULATIONS

Platings on water agar from the decayed kernels commonly yielded *Pythium* sp., which readily was isolated in pure culture. The dozen or more isolates obtained all appeared alike in cultural characteristics and might well have been the same species. A single culture, designated C–2, was selected at random from the various isolates and used in inoculation experiments.

METHODS OF INOCULATION

The pathogenicity of *Pythium* sp., culture C–2, was tested on crown-wounded corn kernels by the two following methods of inoculation used by the writer in seed decay studies.

Sand-Culture Method. Week-old cultures, from mycelial transfers, grown in 100 × 15 cc. Petri dishes on 2 per cent potato-dextrose agar are
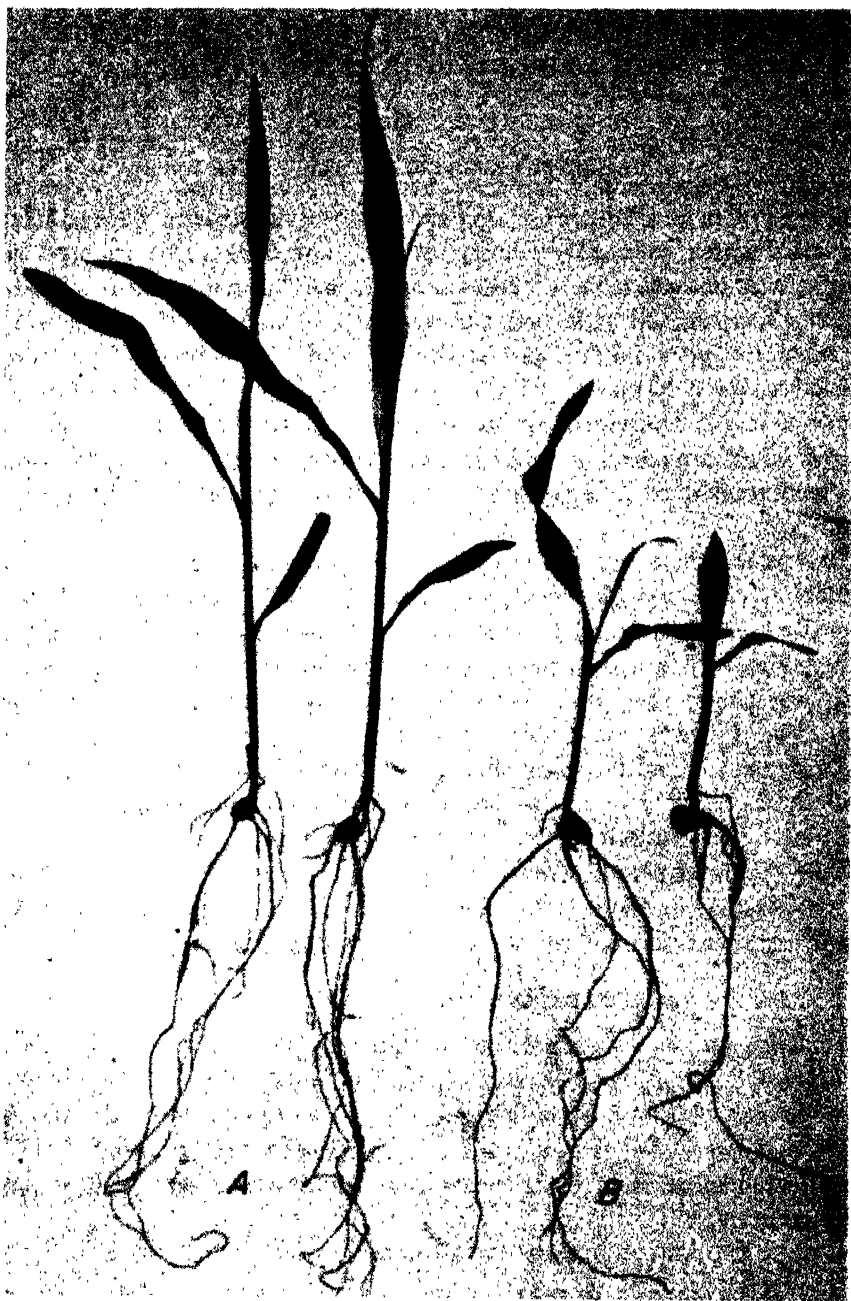
FIG. 1. *A*. Healthy corn seedlings grown in compost soil at 20°–24°C. from crown-wounded kernels that had been dusted with Arasan seed disinfectant. *B*. Stunted but otherwise seemingly healthy corn seedlings from nontreated seed. Not shown clearly in the picture is an agglutination of soil at the wounded surfaces of the nontreated seed. This evidence of fungus invasion of the kernels was absent from those that had been treated.

kneaded by hand with autoclaved white sand, mixing the contents from a single dish (agar and fungus) with approximately 250 cc. of sand. A 6-inch earthenware crock then is about half-filled with compost soil after which are added, in successive order, inch-deep layers of autoclaved sand, inoculated sand, and finally, either autoclaved sand or Vermiculite. The corn kernels are planted in the layer of inoculated sand before the covering layer is added.

Fungus-Mat Method. This method is identical with that described above except that the kernels are planted between fungus mats which have been removed intact from the Petri dishes. This more drastic method of inoculation has certain advantages, particularly for tests at low temperatures with cultures of relatively low virulence.

INOCULATION EXPERIMENTS IN WARM AND COLD SOILS

Crown-wounded kernels from a mixture of two, dent corn hybrids, nontreated and dusted with Arasan, were inoculated with *Pythium* sp., culture C-2, by both methods and grown at controlled warm and cold soil temperatures, with one series of controls in naturally infested compost soil and the other in a layer of sterile sand over compost soil.

In the experiment in which the sand-culture method of inoculation was used, the nontreated and Arasan-dusted seed was planted in four 25-kernel replications for each of the following: (1) seed planted in compost soil; (2) seed planted in the inoculated sand; (3) seed planted in a layer of sterile sand over compost soil. This last served as a dual control, for disease from artificial inoculation and also for possible stimulatory effects from the dust.

The inoculations with the fungus-mat method were made in four 15-kernel replications each of nontreated and Arasan-dusted seed.

In the cold soil experiment the crocks first were kept for 14 days in a walk-in refrigerator maintained at 11° C. and then moved to a warm greenhouse (20°–24° C.) to complete germination and seedling growth. The test in the warm soil was made entirely in the 20°–24° C. greenhouse.

RESULTS

The data from the experiment in the warm and cold soils are summarized in table 1 and the more pertinent aspects of the results are illustrated, in part, in figures 2 and 3.

Seed Decay in Cold Soil

Nontreated seed artificially inoculated by either of the two methods resulted in 100 per cent pre-emergence killing (typical seed decay) when kept for 14 days at 11° C. (Fig. 2, A, a). Likewise, nontreated seed planted in compost soil also resulted in no seedling emergence under these conditions. In contrast, seed that had been dusted with Arasan produced good stands of mostly healthy seedlings both in the artificially inoculated sand (Fig. 2,

FIG. 2. Effects of soil temperature on symptoms when crown-wounded corn kernels had been inoculated (fungus-mat method) with *Pythium* sp. *A*. Effects at 11°C.: *a*. pre-emergence killing of nontreated seed, *b*. healthy plants from Arasan dusted seed. *B*. Effects at 20°–24°C.: *a*. stunting without conspicuous lesions on mesocotyls or roots from nontreated seed, *b*. healthy seedlings from kernels treated with Arasan. Results nearly identical with those shown here were obtained following inoculations with the sand-culture method.

A, b) and in naturally infested compost soil. The efficacy of Arasan was impaired perceptibly when the treated kernels were subjected to the drastic fungus-mat method of inoculation, but even with this, the seed treatment resulted in nearly 75 per cent stands of healthy seedlings.

TABLE 1.—*Effects of high and low soil temperatures on disease symptoms caused by Pythium sp. in corn seedlings when nontreated and Arasan-dusted injured seed was planted in naturally infested and artificially inoculated soils and in autoclaved sand*

Source of disease	11°C.			20°–24°C.		
	Stand	Stunted seedlings	Control index[a]	Stand	Stunted seedlings	Control index[a]
	Pct.	Pct.		Pct.	Pct.	
Natural infection (compost soil)						
Nontreated seed	0		0	98.0	59.1	69.0
Arasan-dusted seed	90.0	4.4	88.0	98.0	16.3	90.0
Sand-culture inoculations						
Nontreated seed	0		0	96.0	56.3	69.0
Arasan-dusted seed	87.0	7.0	84.0	100	2.0	98.0
Fungus mat inoculations						
Nontreated seed	0		0	86.7	100	43.3
Arasan-dusted seed	73.3	0	73.3	93.3	0	93.3
Autoclaved sand (control)						
Nontreated seed	91.0	6.6	88.0	100	7.0	93.3
Arasan-dusted seed	94.0	5.3	91.5	100	2.0	98.0

[a] Theoretical range of control is from 0 (no seedlings emerged) to 100 (perfect stand of all normal-sized plants). Stunted seedlings were given half the numerical values assigned the healthy plants in calculating the control index.

Both nontreated and Arasan-dusted seed produced nearly identical stands of mostly healthy seedlings when the kernels were planted in the noninoculated layer of autoclaved sand in the low temperature experiment. This proved that the seed decay that resulted from artificial inoculation and from sources of natural infection in the compost soil could not be attributed to seed-borne infections.

Stunted Seedlings in Warm Soil

The principal effect of 20°–24° C. when nontreated seed was subjected to inoculation with *Pythium* sp., culture C–2, or when it was planted in naturally infested compost soil, was a pronounced stunting in the seedlings (Fig. 2, B, a and 3, A, a).

The fungus-mat method of inoculation again proved the more drastic in the warm soil with the control index for nontreated seed very significantly lower than for the other sources of infection. This was due partly to a slight amount of pre-emergence killing, but mainly because every seedling from the nontreated seed was stunted.

The results from the sand-culture inoculations more nearly simulated

the disease effects when nontreated seed was exposed to natural infection
in the compost soil   Here the control indices were identical   Neither

FIG 3   *A , a* Stunted seedlings from nontreated seed, *b.* healthy plants from Arasan
dusted seed when crown wounded corn kernels were planted in compost soil at 20°-24°C.;
B , a. and *b* same as *A* except kernels were planted in a layer of noninoculated, autoclaved
sand over the compost soil

source of infection caused any appreciable amount of pre-emergence killing
and both resulted in about two-thirds of the seedlings becoming stunted
(Fig. 3, A).

Arasan treatment of seed subjected to artificial inoculation resulted in

almost perfect control of disease at the higher temperature. Some stunting (16 per cent) recorded for treated seed planted in the compost soil might have been due to the presence of more virulent forms of *Pythium* in the naturally infested soil than the pure culture used in the artificial inoculations.

Only a few stunted seedlings (7 per cent) were observed among those from kernels that had germinated in the layer of autoclaved sand (Fig. 3, B). This demonstrated rather conclusively that the main stunting effects in these experiments were caused by the soil fungi and not by seed-borne parasites. It is very likely that the 7 per cent of stunting mentioned was caused by seed infections because platings of the seed disclosed some seed infections with miscellaneous fungi some of which probably were capable of causing stunting at the higher temperatures.

DISCUSSION

The belief commonly held that disease-free seed corn is not attacked by *Pythium* or other seed-decay types of fungi, except in cold wet soils, is disproved in these experiments. Severe stunting in seedlings from nontreated, crown-wounded kernels was demonstrated at soil temperatures very favorable for germination and seedling growth (20°–24°C.) in naturally infested soil and also following pure culture inoculations with *Pythium* sp., culture C-2. This fungus, originally isolated from a stunted seedling grown in warm soil, was proved similar to the seed-decay types in artificial inoculations in cold, wet soils where it caused 100 per cent pre-emergence killing in nontreated seed.

Whether the stunting caused by *Pythium* sp., culture C–2, in warm soils is typical of what might be expected from other seed-decay fungi under similar circumstances has not been investigated. In view of the unspecialized type of parasitism involved in the destruction of endosperm and scutellum tissues, it would seem probable that other seed-decay fungi would behave similarly. The artificial methods of inoculation described herein provide a ready means for a study of this question, and also for a much-needed investigation of soil fungi in general, with respect to their disease potentialities in germinating seed corn.

The excellent protection against stunting in warm soil afforded by Arasan seed treatment in the present studies indicates that benefits from seed treatment with this and other efficacious dusts occur in most field plantings, regardless of how favorable the temperatures during the germination period. Despite improved methods of processing seed corn to reduce the amount of mechanical damage, many injured kernels may still be found in most commercial seed lots.

The present study illustrates an advantage of investigating the complicated corn seedling disease problem in singling out an individual variable from the many involved in the complex and then studying that one in "accentuated" tests where every kernel, theoretically at least, responds

alike when subjected to experiment in a controlled environment. The disease in the warm soil described herein would not have been easily detected, much less interpreted correctly, in experiments with ordinary samples of seed.

SUMMARY

Stunting in corn seedlings without conspicuous lesions on mesocotyls or roots, first observed when nontreated nearly disease-free kernels with crown wounds were planted in warm (20°–24°C), compost soil, was demonstrated at this temperature following pure culture inoculations with *Pythium* sp. This fungus, originally isolated from a naturally infected seedling grown in warm soil, caused 100 per cent seed decay in inoculations of nontreated seed at 11°C.

Arasan seed treatment afforded excellent control against stunting in both naturally infested and artificially inoculated soils. The benefits demonstrated in the warm soil are an advantage of corn seed treatment heretofore unrecognized.

Two methods of pure culture inoculation with *Pythium* sp. are described.

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EVIDENCE OF THE NON–EXISTENCE OF PHYSIOLOGICAL RACES IN CRONARTIUM RIBICOLA

GLENN GARDNER HAHN[1]

Apropos of Mielke's (6) fairly recent and brief discussion of physiological races or strains of white-pine blister rust, which is caused by *Cronartium ribicola* A. Fisch., it is desirable at this time to present a summary of certain accumulated data relating to the Viking red currant (2). Its long history of freedom from the establishment of the pine parasite upon its leaves, serves to support the view held by some pathologists that there is only one race in *C. ribicola*.

Although the new varietal name, Viking (2), was published in the mid-30's, the red currant itself is not new. When the rust-immune Viking (syn. Rød Hollandsk Druerips, Holländische Rote), with probable other synonyms, was introduced specifically for blister-rust investigations in the early 1930's into the United States from Norway, it was deemed advisable to report the foreign introduction under a new name in order that it would not be confused with our domestic rust-susceptible red currant variety, The American Red Dutch (2). Viking, a *Ribes petraeum* Wulf. hybrid, actually is a very old variety and has been known in the horticultural literature for at least a century and probably very much longer. Under the name, Rød Holländsk Druerips (2), the currant has been grown in Scandinavia for many years in gardens and commercial plantations; as Holländische Rote it has been cultivated extensively on the European Continent as well, particularly in Holland, Germany, and Switzerland. Accordingly, the variety has been exposed to blister-rust infection over wide areas in western Europe for many years. There are no records (5, p. 345), however, of *Cronartium ribicola* having been observed upon its leaves. The late eminent forest pathologist, Tubeuf (7), working independently, tested Holländische Rote at Munich, Germany, and reported it to be immune from *C. ribicola*. The following decade the writer reported stock of Holländische Rote from the Grafrath Experiment Station, Munich, as being, as Tubeuf had recorded, immune from the rust (5). Field tests later showed the German introduction to be identical morphologically with the Viking stock from Norway.

Before the writer undertook American investigations, he tested Viking brought from Norway into Great Britain with *Cronartium ribicola* collected in Scotland and found the red currant to be immune (1; 2, p. 9; 5, p. 343). In this respect in accordance with the writer's concept of the term im-

munity, it implies an invasion of Viking leaves by germ tubes of *C. ribi-cola* at certain stages of leaf development, but as previously indicated the infectious agent is unable to establish itself (5, p. 343). Subsequently the writer demonstrated under conditions of supervised testing in the United States that the introduced variety was immune when exposed to natural infection of *C. ribicola* in the East in New England (Maine, New Hampshire, Massachusetts, and Connecticut) and in New York State, and in the West in Oregon (3).

In Canada, similar stock (3, p. 861) of the variety provided by the United States Department of Agriculture was tested independently by Canadian pathologists and horticulturists at the Central Experimental Farm, Ottawa. These workers were unable to find any establishment of *Cronartium ribicola* on leaves of Viking although nearby plants of varieties of *Ribes nigrum* L. became heavily infected with the rust (5, p. 347).

In addition to these supervised field experiments, large plantations of Viking, which was also shown by the writer to be nearly homozygous for rust resistance (4), were established for horticultural testing by Professor W. H. Alderman in 1936 in Minnesota at the North Central Experimental Station at Grand Rapids and at the Fruit Breeding Farm of the University of Minnesota near Excelsior. This stock, like that in the foregoing tests mentioned, had been propagated from cuttings introduced from Norway (3, p. 861) and distributed by the United States Department of Agriculture. According to information transmitted by Professor Alderman, the currant has done very well and has been free of rust in white-pine (*Pinus strobus* L.) territory where Viking, with the approval of the local blister-rust authorities, is the only red currant grown.

On the basis of experience with cereal rusts in which different races are known to occur, it might be anticipated that more than one race of *Cronartium ribicola* exists, and, if so, one of them might be capable of becoming established upon Viking. However, results with the variety in North America together with observations and tests on it in Europe have not revealed the establishment of blister rust upon its leaves. If there are races of *C. ribicola*, it has been suggested that Viking may react the same to all of them. Moreover, if different physiologic races of the pine parasite actually exist, experience with Viking over many years in Europe and North America has not indicated their presence.

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The Dissemination of Xanthomonas malvacearum by Irrigation Water.
—Wind-blown rain and droplets of splashed water from fog and dew have
long been recognized as disseminating agents in the spread of angular leaf
spot of cotton.[1,2] Evidence is presented here which indicates that surface
irrigation water may also act as a disseminating agent.

During the course of a seed treatment experiment at Sacaton, Arizona,
in 1938, two plots of Pima cotton seedlings, *Gossypium barbadense* L., be-
came severely affected with angular leaf spot. The disease appeared shortly
after the first flood irrigation, which was applied May 2. A previous dis-
ease survey of these plots had shown no angular leaf spot, although a plot
lying 15 feet above them in the same irrigation border had approximately
10 per cent infected plants. By May 19, the two plots that at first had
been disease-free had 67.5 per cent of 891 seedlings infected in one instance
and 46.0 per cent of 1480 seedlings infected in the other.

As there had been no rain to account for the spread of the bacterium,
the irrigation water was believed to be the disseminating agent. To test
this hypothesis, a nearby disease-free planting of Pima cotton seedlings was
divided into three parts, designated plots 1, 2, and 3, and bordered so that
each could be flood irrigated independently. On May 23, irrigation water
was applied to plot 2 after it had passed through plots that were infested
with angular leaf spot. The distance between the diseased plot and the
disease-free plot was about 15 feet. Plots 1 and 3 were irrigated at the
same time with water from lateral ditches that had no opportunity to con-
tact cotton seedlings. A census on June 14, showed 44.2 per cent infection
in 486 seedlings in plot 2, whereas plots 1 and 3 with a combined total of
889 seedlings remained free of the disease. In this instance also there
had been no rain. Thus the results were in agreement with the earlier
observations.[3]

In 1943 both surface irrigation and sprinkling were effective in dis-
seminating *Xanthomonas malvacearum* (E. F. Sm.) Dowson. This was
demonstrated during studies on the winter carry-over of angular leaf
spot. A previously diseased crop of the upland variety Acala (*Gossypium
hirsutum* L.) was used as a source of primary infection, while a disease-free
planting of the American-Egyptian variety S × P (*G. barbadense* L.) was
used to determine the spread of the disease. Both are susceptible to an-
gular leaf spot and the seedlings of each may be readily distinguished upon
close examination.

The test was conducted as follows: Acala bolls were collected in Feb-

[1] Faulwetter, R. C. Dissemination of the angular leafspot of cotton. Jour. Agr. Res.
[U.S.] 8: 457–475. 1917.
[2] Faulwetter, R. C. Wind-blown rain, a factor in disease dissemination. Jour. Agr.
Res. [U.S.] 10: 639–648. 1917.
[3] Reference was made to this experiment in the following report: King, C. J. and
E. B. Parker. Angular leaf spot of cotton in the irrigated valleys of Arizona and New
Mexico. U. S. Dept. Agr., Pl. Dis. Reptr. 23:32. 1939.

ruary from a field that had had angular leaf spot the previous season. In March the bolls were spread across the upper end of a plot of land that had not been cropped with cotton for several years. The soil was plowed, irrigated, and disked; and on March 23 it was planted with seed known to be free of *Xanthomonas malvacearum*. A good stand was obtained


LEGEND

■ INFECTED VOLUNTEER ACALA

□ NON-INFECTED VOLUNTEER ACALA

● SRINKLED AREA

● INFECTED PLANTED SXP

○ NON-INFECTED PLANTED SXP
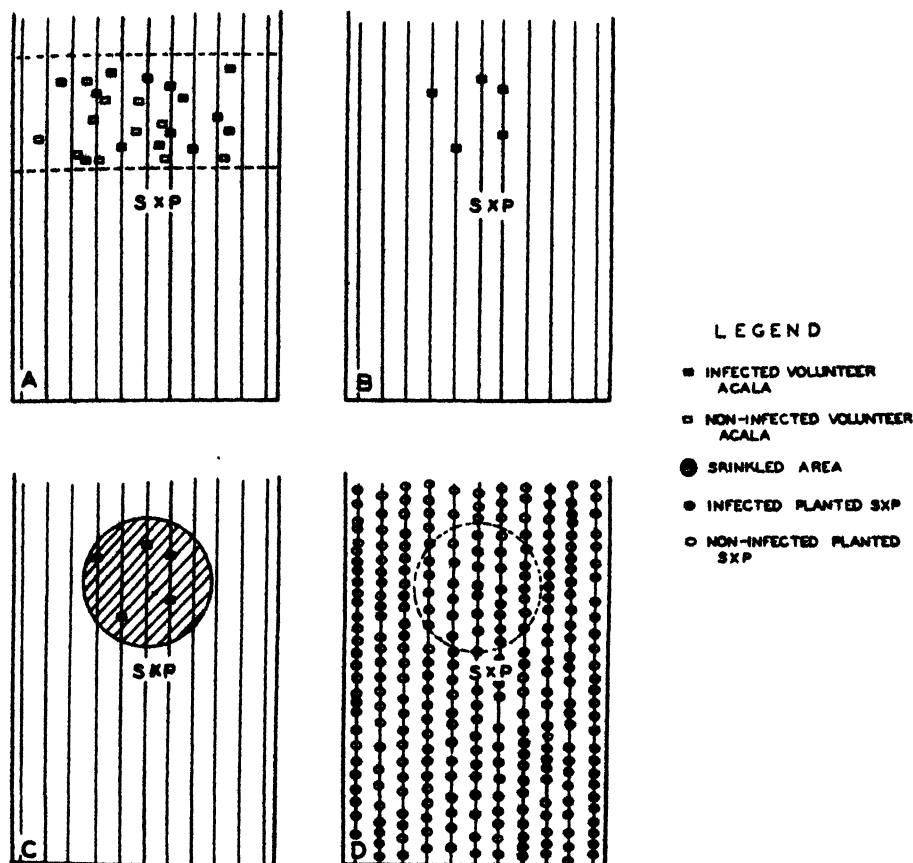
FIG. 1. Maps showing the spread of angular leaf spot from diseased volunteer Acala seedlings to disease-free planted S×P seedlings. A. Infected and non-infested Acala seedlings as they occurred among the planted rows of S×P. B. Infected Acala seedlings remaining in the S×P seed rows after cultivation. C. Sprinkled area in which a majority of the S×P seedlings became infected. D. Thinned stand of S×P seedlings showing infected and non-infected plants following flood irrigation.

from the planted seed and a small percentage of the buried Acala bolls produced seedlings. The plot was mapped on April 27 (Fig. 1, A). Approximately one-half of the clumps of Acala seedlings had angular leaf spot, but no infection was found in the S×P seedlings. A cultivation on May 11 removed all of the clumps of Acala seedlings except those in line with the seed rows. Five clumps with infected seedlings remained (Fig. 1, B). On May 12, a rotating lawn sprinkler was placed in the

portion of the plot in which the diseased Acala seedlings were located. Within 10 days, a majority of the S × P seedlings within the sprinkled area had angular leaf spot lesions. The affected area of the plot coincided with the sprinkled area (Fig. 1, C), while all the seedlings outside remained disease-free. On May 22, the entire plot was flood irrigated, the seedlings being partially submerged for about 1 hour. The stand was thinned to approximately 15–inch spacings on June 2, without regard to angular leaf spot infection, and the entire plot was then mapped for diseased seedlings.

Angular leaf spot was then found below and immediately to the sides of the sprinkled area (Fig. 1, D) where approximately 50 per cent of the thinned stand was affected, while the upper portion of the plot and the outside rows remained disease free. Thus the disease had spread in the same general direction as the flow of the irrigation water. Infected seedlings showed that the pathogen had been carried in two instances to the end of the plot, a distance of 25 feet. Again there was no rain between the time of planting and final inspection of the plot.—C. J. KING, late Senior Agronomist, and L. A. BRINKERHOFF, Associate Pathologist, United States Field Station, Sacaton, Arizona.

A Greenhouse Bench Moist Chamber for Large Scale Inoculations.[1]—In the course of a breeding program, it is frequently desirable to test large numbers of plants for resistance to specific diseases under controlled conditions. For many diseases, a high humidity for a given period is necessary to induce infection. However, the desired humidity is frequently difficult to achieve in an ordinary greenhouse without a moist chamber. During the past two years, a. moist chamber of large capacity and simple design has been in operation at the U. S. Regional Pasture Research Laboratory. The chamber has a capacity of 26 flats (12 × 24-inch) or 400 pots (4-inch) and can be constructed for less than $100.00. It may be made either portable or permanent over a greenhouse bench. The moist chamber and humidifying mechanism are shown in figure 1.

In erecting the framework, 2 × 2-inch uprights were fastened at each inside corner of the bench. Wooden braces were secured to the uprights and wires were stretched the length of the bench and attached to the uprights. Another wire was fastened to the greenhouse frame to support the peak of the roof for the chamber. Plastic shower curtain material, clear Parafilm,[2] was then spread over the wires to form a canopy. Strips 15 feet long and 3 feet wide were lapped about two inches at the borders and were long enough so that the ends reached below the sides of the bench. Each strip

thus formed a panel that was easily folded back to afford access to plants at any place in the chamber. The ends of the chamber were fashioned of the same material, cut to fit and tacked to the wooden brace between the uprights. The upper portions were pinned to the nearest canopy panel. At one end of the chamber a hole was cut in the Parafilm to allow introduction of moisture from the humidifier.
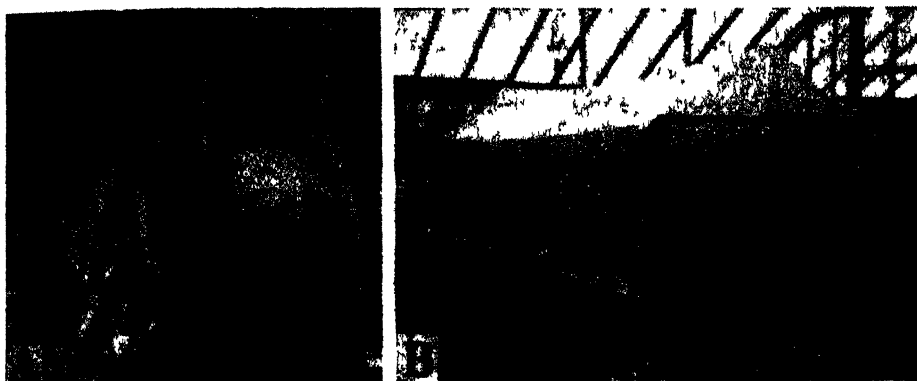

FIG 1. A. Close up view of humidifying apparatus. B. Moist chamber erected over a greenhouse bench.

A Model 42 humidifier manufactured by the Standard Engineering Works, Pawtucket, Rhode Island, was used. The unit draws water up the outer surface of an enclosed cone rapidly rotated by an electric motor and throws it against the vanes of a circular grid where it is broken into a fine mist and blown into the chamber through an adjustable elbow. A float valve attached to a water line regulates the water level in the reservoir of the humidifier. At present, the unit is operated satisfactorily by an automatic time clock that turns the humidifier on and off 15 minutes out of every hour from 7:00 a m to 7:00 p.m. It has not been found necessary to operate the unit at night.

The artificial fog is circulated to all parts of the chamber within one minute after the humidifying unit starts to operate. The moisture particles are small and settle slowly and uniformly, thus covering leaves of plants within the chamber with a film of moisture resembling dew. The Parafilm canopy admits sufficient light to prevent etiolation of plants even when kept in the chamber for 10 to 14 days. It is mildewproof, resists tearing, and to date shows no sign of deteriorating. The chamber has operated satisfactorily in large-scale inoculation tests with *Sclerotinia trifoliorum* on forage legumes. Excellent infection has also been obtained with such foliar diseases as *Puccinia coronata* on *Festuca elatior* and *Stemphylium sarcinae-forme* on *Trifolium pratense.—*K. W. KRETLOW, U. S. Regional Pasture Research Laboratory, State College, Pennsylvania.

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A METHOD FOR PRODUCING ARTIFICIAL EPIDEMICS OF DIPLODIA EAR ROT[1]

ARNOLD J. ULLSTRUP[2]

Diplodia ear rot is one of the major diseases of corn (*Zea mays* L.) in many areas where the crop is grown. Wide differences exist among inbred lines in resistance to the disease, but no line is known to be immune from attack. The only feasible means of controlling this disease is by breeding and selecting for resistance. Because of fluctuation in the prevalence of diplodia ear rot from year to year and from one locality to another in a given season it is not often convenient to rely on natural epidemics to determine the relative resistance of host material.

A satisfactory method of producing artificial epidemics of diplodia ear rot could be a useful adjunct to any corn breeding program where resistance to this is to be incorporated into inbred lines.

The present paper reports the development of an inoculation technique that has proved satisfactory over several years.

MATERIALS AND METHODS

Inoculum was increased on sterile, whole oats in 2-quart fruit jars. The jars were filled to about one-half their capacity with oats and an excess of warm tap water added. After 4 hours the water was drained off and 200 ml. of hot tap water added to each jar. The jars were plugged, pre-heated for 15 minutes, and then autoclaved for 3 hours at 15-lb. pressure.

Isolates of *Diplodia zeae* (Schw.) Lev. were selected on the basis of rapid and abundant sporulation as determined from preliminary tests on sterile whole oats in small flasks or as indicated by direct plating of infected kernels on acidified potato-dextrose agar. Except in an experiment where the virulence of different cultures was compared, only one culture was used for a given experiment. Inocula from different isolates were never mixed to make up a composite spore suspension. The same isolate was not always used from year to year.

The sterile oats were seeded with mycelium of the selected isolate and incubated for about 6 weeks in the light at a temperature ranging between 24° and 26° C. The stock spore suspension was prepared by removing the material from the jars to a cheesecloth sack and kneading the mass in water in order to separate the spores from the oats. The suspension was then

filtered through a layer of cheesecloth and made up to volume at a rate of a jar of inoculum in one quart of water. This stock spore suspension from a good sporulating culture (Fig. 1) was very dark and comparable in color to fresh India ink diluted 1:10,000.

The stock suspension was diluted usually at a rate of 2 qt. in 2.5 gal. just prior to spraying on the ears. A simple 2.5-gal. compressed air sprayer was used to apply the spore suspension over the entire ear from
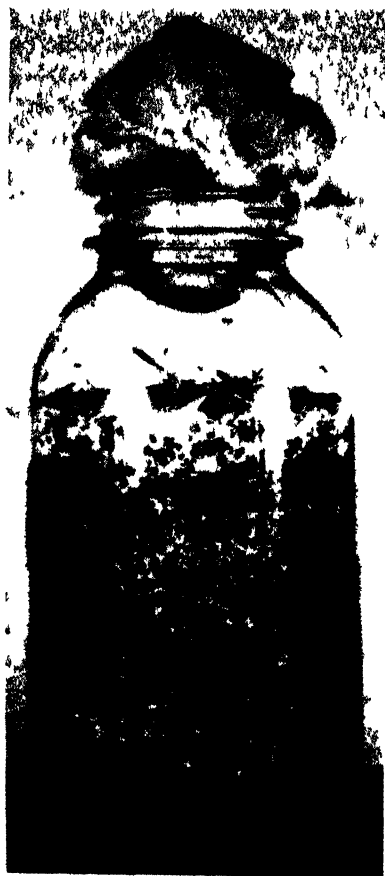
FIG. 1. Fruit jar culture of *Diplodia zeae* after 5 weeks' incubation. The dark areas are composed of masses of pycnidia and pycnospores.

silk to shank. Two and one-half gallons of spore suspension were sufficient to inoculate 250 to 300 plants. Plants were usually inoculated only once, although in some years and in certain experiments two inoculations were made with an interval of 5 to 7 days between applications.

Single cross hybrids were used in all experiments. The relative resistance of an inbred line was determined by comparing the mean amount of disease sustained in all its crosses with the mean amount of disease in any other group of hybrids, within the all-combination set, having another

inbred parent in common. This could be done where inbred lines to be tested were crossed in all possible single cross combinations and compared in a two-way table.

The plots were uniformly 1×10 hills with 3 plants per hill and were replicated 3 to 6 times in a simple randomized block design. Harvests were made usually during the first two weeks in November. Each plot was harvested separately and every ear was inspected in the laboratory. If no disease was observed on the surface, several kernels were removed from points well distributed over the ear to determine the extent of infection, if any, at the kernel tips.

The amount of disease in each plot was expressed as the percentage of *Diplodia*-infected ears; or the percentage by weight of *Diplodia*-infected kernels in a representative 250-gram sample of the shelled corn from an entire plot; or by a disease index calculated by the following formula:

$$\frac{20 \ (nC_0 + nC_1 + nC_2 + nC_3 + nC_4)}{N}$$

where n = the number of ears in each class; C_0, C_1, C_2, C_3, C_4 = the class values (0 to 4) for healthy, one-fourth rotted, one-half rotted, three-fourths rotted, and completely rotted ears, respectively; N = the total number of ears in an entire plot. The disease index measured the amount of disease, and weight was given to the extent of rotted area on each ear.

EXPERIMENTAL RESULTS

Influence of time of inoculation. Inoculations were made at the time of full silk or within a week thereafter and continued at weekly intervals for 4 or 5 weeks. A single plot was inoculated only once. Both resistant and susceptible single cross hybrids were used. Inoculum was made up periodically to insure that spores were approximately the same age for all inoculations. The hybrids and different dates of inoculation were randomized independently within each replication. All plots had been planted on the same date.

The greatest amount of infection and the clearest differential among hybrids were secured in those plots inoculated within a week or two after full silk. There was a slight, but non-significant, trend toward a decrease in disease from the first inoculation to that made 3 weeks after full silk. Inoculations made 4 or 5 weeks after full silk resulted in a small amount of ear rot. An example of the difference in percentage of rotted ears sustained from early as compared with late inoculations is shown in table 1.

Effect of dilution of inoculum on the incidence of ear rot. A stock spore suspension, made up at a ratio of one 2-qt. jar of culture per quart of water, was used at rates of 0.5, 1.0, 2.0, and 3.0 qt. stock suspension in 2.5 gal. water. Hybrids and different concentrations of inoculum were randomized independently within each replication.

Even the lowest dilution of 0.5 qt. stock suspension per 2.5 gal. of final

TABLE 1.—*Effect of time of inoculation on the incidence of diplodia ear rot. Early inoculations made about 1 week after full silk*

Single crosses	Time of inoculation	Mean percent-age of *Diplodia*-rotted ears	Minimum significant difference	
			1 per cent level	5 per cent level
		Per cent	Per cent	Per cent
Tr × Hy	Aug. 8, 1942	46.2	11.2	7.2
do.	Sept. 5, 1942	23.7		
Hy × 38–11	Aug. 11, 1942	24.7	11.9	7.6
do.	Sept. 8, 1942	7.8		
Wf9 × R4	Aug. 6, 1942	11.6	10.7	6.8
do.	Sept. 5, 1942	2.4		
Tr × Hy	Aug. 7, 1944	77.4	23.0	14.7
do.	Sept. 4, 1944	48.2		
M14 × Hy	Aug. 7, 1944	50.8	25.8	16.5
do.	Sept. 4, 1944	9.5		
540 × 90	Aug. 7, 1944	41.7	15.0	9.5
do.	Sept. 4, 1944	4.3		
R4 × 90	Aug. 7, 1944	26.3	21.1	13.4
do.	Sept. 4, 1944	5.9		

inoculum was sufficient to give a good differential between hybrids (Table 2). Significant differences in percentage of rotted ears are shown between certain dilutions of inoculum when applied to the more susceptible single crosses. The difference between dilutions, however, is not consistently maintained in the resistant hybrids.

Comparison of different isolates of Diplodia zeae. Isolations of *Diplodia zeae* were made from 5 collections of diseased ears gathered from widely separated geographical locations. Each of the 5 isolates was seeded separately on sterile whole oats in the usual manner. Four single crosses were used as host testers, two of which were known to be susceptible and two were known to be relatively resistant. Ears were inoculated 10 days after full silk and again 7 days later. Isolates and hybrids were randomized independently within each replication. Particular care was taken to prevent any mixing of inoculum.

TABLE 2.—*Effect of dilution of inoculum on the incidence of Diplodia-rotted ears*

Single crosses	Mean percentage of *Diplodia*-rotted ears after inoculation with indicated stock spore suspension in 2.5 gal. final inoculum				Minimum significant difference between dilutions	
	0.5 qt.	1.0 qt.	2.0 qt.	3.0 qt.	1 per cent level	5 per cent level
					Pct.	Pct.
Tr × M14	56.3	65.5	58.8	69.5	13.7	7.6
RR98 × M14	40.9	47.4	48.0	66.1	5.1	2.8
RR98 × R4	2.1	3.8	0.8	5.4	7.8	4.0
540 × R4	2.1	3.7	1.8	3.2	5.0	2.7
Minimum significant difference between hybrids:						
1 per cent level	3.0	4.4	8.7	6.2		
5 per cent level	1.7	2.5	4.8	3.4		

TABLE 3.—*Incidence of ear rot induced by different isolates of Diplodia seae. Each single cross inoculated twice with a given culture*

Single crosses	Mean percentage of Diplodia-rotted ears from cultures					Minimum significant difference	
	No. 2	No. 6	No. 9	No. 13	No. 23	1 per cent level	5 per cent level
						Pct.	Pct.
Tr × M14	78.2	92.2	91.1	83.6	84.3	13.3	9.5
Tr × Hy	71.8	89.8	85.1	84.5	71.4	12.2	8.7
R4 × 90	17.3	35.2	38.0	13.4	16.1	21.3	15.2
Wf9 × R4	15.6	31.0	29.0	20.7	17.4	13.2	9.4

There was no indication of differential virulence among the cultures with the host materials used (Table 3). Plots inoculated with cultures No. 6 and No. 9 had somewhat more disease than those inoculated with the other cultures. The reason is not known, although there may have been somewhat better sporulation or a higher percentage of viability of the spores.

Indexing of inbred lines. Inbred lines of corn were indexed for resistance to diplodia ear rot for several years within groups combined in systematic single crosses. The object was to determine both the relative resistance of the lines to the disease and the uniformity of disease reaction from year to year. The results of these tests are shown in tables 4 and 5. The inbred lines Tr and M14 have usually contributed high susceptibility to crosses. In some years M14 has had greater susceptibility than Tr but in other years the opposite was true. None of the other inbred lines tested has contributed so much susceptibility to crosses. The inbred lines 540, R4, and 90 invariably have been the lines that contributed the most resistance to their crosses. The inbred lines 38–11, Hy, and Wf9 usually have been of intermediate rank in contributing susceptibility to their crosses.

TABLE 4.—*Mean percentages of Diplodia rotted ears in 45 hybrids involving 10 inbred lines in all possible single cross combinations. Inoculations made in 1943*

	M14	Tr	A	Wf9	Hy	38–11	66	R4	90	540	Means for inbreds
M14		56.5	79.2	46 5	34.0	36.2	29.1	26.4	24.6	27.0	41.1
Tr	56.5		29.6	42 0	42.4	30.7	30 9	20.9	21.1	9.4	31.5
A	79.2	29.6		17.1	6.3	24.4	21.3	20.0	10.0	5.1	23.7
Wf9	46 5	42.0	17.1		11.5	11.5	15.5	1.9	12.7	6.8	18.4
Hy	34.0	42.4	6.3	11.5		17.9	6.7	24.1	11.2	10.8	18.3
38–11	36.2	30.7	24.4	11.5	17.9		7.1	11.7	10.4	9.4	17.7
66	39.1	30.9	21.3	13 5	6.7	7 1		9.4	6 7	5.3	15.8
R4	26.4	20.9	20.0	1.9	24.1	11.7	9.4		11.1	6.8	14.7
90	24.6	21.1	10.0	12.7	11.2	10.4	6.7	11.1		3.3	12.3
540	27.0	9.4	5.1	6.8	10.8	9.4	5.3	6.8	3.3		9.3

Minimum significant difference for inbred means:
4.8 per cent at 1 per cent level.
3.6 per cent at 5 per cent level.

TABLE 5.—*Mean percentages of Diplodia-rotted ears in 28 hybrids involving 8 inbred lines in all possible single cross combinations. Inoculations made in 1947*

	M14	Tr	38–11	Wf9	Hy	R4	540	90	Means for inbreds
M14		86.5	77.1	78.1	77.9	72.1	71.0	32.8	70.8
Tr	86.5		84.9	91.8	69.1	42.9	59.4	58.6	70.5
38–11	77.1	84.9		78.2	41.0	35.2	24.2	43.9	54.9
Wf9	78.1	91.8	78 2		32.2	14.6	35.2	24.6	50.7
Hy	77 9	69.1	41.0	32.2		43.6	31.6	30.4	46.5
R4	72.1	42.9	35 2	14.6	43.6		6.4	28.3	34.7
540	71.0	59.4	24.2	35.2	31.6	6.4		13.9	34.5
90	32.8	58.6	43.9	24.6	30.4	28.3	13.9		33.2

Minimum significant difference for inbred means:
    6.6 per cent at 1 per cent level.
    5.0 per cent at 5 per cent level.

Methods of recording data. The amount of ear rot in each plot was recorded as: (1) percentage of *Diplodia*-rotted ears; (2) percentage by weight of *Diplodia*-rotted kernels in a representative 250-gm. sample of the shelled corn; and (3) the disease index. In a number of experiments, two of the three records were used. The coefficients of correlation between these records were calculated and are shown in table 6. The extremely high values for r in different years and when different host materials were used suggests that the three methods of recording disease incidence are equally satisfactory.

DISCUSSION

The success and usefulness of a method for inoculating corn ears with *Diplodia zeae* depend on several criteria: (1) a clear differentiation between resistant and susceptible host materials; (2) disease reactions of inbred lines and hybrids comparable to those experienced under natural epidemics of the disease; (3) reproducibility of results from year to year, and no great effect of environment on the relative disease reactions of the host plants; (4) simplicity of execution and freedom from complicated and laborious procedures; and (5) simulation of those phenomena attending the natural mode of infection by the parasite. *D. zeae* is not a wound para-

TABLE 6.—*Correlations between percentage by weight of Diplodia-rotted kernels and percentage of Diplodia-rotted ears and between Diplodia-rotted kernels and disease index. Correlations based on the mean values for single crosses*

Year	Number of single crosses observed	Number of replications on which means values are based	Variables correlated	Value of r
1941	15	3	Rotted kernels *vs* rotted ears	0.99
1942	15	6	do. *vs* do.	0.99
1943	21	5	do. *vs* do.	0.95
1943	21	5	do. *vs* disease index	0.98

site, and therefore it is conceivable that wounding could nullify effects of barriers, either structural or physiological, that function under normal conditions to impede the entrance of the fungus into the host. Young[3] has described an inoculation method in which toothpicks, over which mycelium of the parasite had been allowed to grow, are inserted into the ears or stalks of corn. Good differentiation between resistant and susceptible host materials are claimed for this method. The inoculation technique described in the present paper amends natural epidemics only in providing for a greater volume and more uniform distribution of inoculum.

The most critical factor involved in the described method is the large scale production of an adequate supply of spores for inoculation. Experiments on dilution of inoculum have shown that a comparatively wide range of spore concentration can be used to obtain a high level of infection. If a poorly sporulating culture is used, the concentration of spores may be too low to obtain sufficient infection for differentiation. Preliminary tests of the sporulating ability of isolates provides for selection of prolific spore producers. A satisfactory culture, when incubated in the light for 4 to 6 weeks at temperatures ranging between 24° and 26° C., produces a large number of spores with a high percentage of germination.

In experiments reported above, the best differential infections were obtained when inoculations were made within a period extending from a few days after full silk until about 2 weeks later. Inoculations made 4 weeks after full silk or later have invariably resulted in appreciably less ear rot. The lower incidence of ear rot following later inoculations may be due to environmental conditions in the late summer unfavorable to the development of diplodia ear rot. Another possible explanation is that there is a shorter period for disease development between inoculation and harvest. The fact that late inoculations have uniformly resulted in less disease even in years when temperatures and humidities were ideal for infection casts some doubt on the possibility that meteorological conditions are responsible for this difference in disease incidence. The possibility that a shortening of the period for disease development accounts for less ear rot following late inoculations is refuted by the observation of rotted ears within two weeks after early inoculations, which is considerably less time than that elapsing between late inoculations and harvest. The most logical explanation of the reduction in ear rot following late inoculations is that the ears become more resistant as they approach maturity. If a block of one single-cross could be inoculated at one time when different plots ranged in stage of development from full silk to 5 weeks after, the question could be solved more precisely. A number of practical difficulties are involved in making plantings at different dates in the spring in order to obtain a situation where such wide stages of maturity exist in an experimental block of one single-cross at the time of inoculation.

[3] Young, H. C. Jr. The toothpick method of inoculating corn for ear and stalk rots. (Abstr.) Phytopath. 33: 16. 1943.

Throughout these experiments, single-crosses have been used and the contribution of the component inbred lines to resistance or susceptibility determined by arranging the data on two-way tables. It is possible that inbred lines, as such, could be used, but confounding factors such as lack of good "fill" of the ears, frequently found in inbred lines, greater susceptibility to other ear diseases, smaller ears, and generally reduced vigor of the entire plant would be encountered.

The amount of diplodia ear rot in each inoculated plot was recorded as percentage of *Diplodia*-infected ears; as percentage by weight of *Diplodia*-infected kernels in a representative 250-gm. sample of shelled corn; or as a disease index. The results of these three methods have been shown to be so closely correlated that the simplest method, namely, that in which the percentage of *Diplodia*-rotted ears is recorded, appears to be adequate. Hoppe and Holbert[4] did not find high correlation coefficients between percentage of rotted ears and percentage by weight of rotted kernels. These authors, however, included all ear rots under conditions of natural infection where the level of disease incidence was appreciably lower than in the experiments reported in the present paper. The exceptionally high correlation coefficients reported here may be due in part to the high incidence of disease and in part to the fact that many ears had incipient infections that were not apparent on the surface. The fact that mean values were used rather than the values of individual plots would also increase the magnitudes of the coefficients of correlation.

A satisfactory method of inoculating corn ears with *Diplodia zeae* could be applied to corn improvement in several ways: (1) Relative resistance of inbred lines to diplodia ear rot could be determined. (2) Heavy epidemics of the disease in segregating populations would permit selection for resistance to diplodia ear rot. (3) Studies of the inheritance of resistance or the nature of resistance to this disease would be facilitated.

SUMMARY

A method for inoculating corn ears with *Diplodia zeae* for differentiating reaction to diplodia ear rot is described.

Large volumes of inoculum were produced on moist sterile whole oats incubated in the light for 4 to 6 weeks at 24° to 26° C. It is important to select isolates that sporulate abundantly and rapidly. Spores for the stock spore suspension were separated from the oat substrate by placing the entire culture from a fruit jar in a cheesecloth sack and kneading the mass in water. The suspension was filtered through cheesecloth and made up to a standard volume at the rate of one jar of culture per quart of water. The stock spore suspension was diluted prior to inoculation at a rate of 2 qt. made up to 2.5 gal. with water. The inoculum was sprayed on the ears, from silk to shank, with a compressed air sprayer of 2.5-gal. capacity.

[4] Hoppe, Paul E. and James Holbert. Methods used in the determination of relative amounts of ear rot in dent corn. Jour. Amer. Soc. Agron. 28: 810–819. 1936.

Two and one-half gallons of spray were sufficient to inoculate 250 to 300 ears.

A high incidence of infection and clear differential effects were obtained when ears were inoculated during a period from a few days after full silk until about two weeks later. Inoculations made 4 or 5 weeks after full silk invariably resulted in a small amount of ear rot.

The stock spore suspension diluted at rates ranging from 0.5 qt. to 3 qt. in 2.5 gal. of final inoculum gave good infection and differential effects.

Experiments with five isolates of *Diplodia zeae*, obtained from ears collected in widely separated geographical areas, failed to demonstrate instances of differential virulence, although some isolates appeared to be somewhat more virulent than others.

Three methods were used to record the incidence of ear rot in inoculated plots. The results of all three methods were very closely correlated. The simplest method is that in which the percentage of rotted ears is recorded.

The inoculation technique gave consistent results when used to index inbred lines for disease reaction. The relative resistance of inbred lines was determined from the results of inoculating all-combination sets of single crosses.

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A COMPARISON OF GROSS PATHOGENIC EFFECTS OF PYTHIUM GRAMINICOLA, PYTHIUM DEBARYANUM, AND HELMINTHOSPORIUM SATIVUM ON SEEDLINGS OF CRESTED WHEATGRASS[1]

W. F. BUCHHOLTZ

As a means of conserving and building soil, farmers in central South Dakota attempt to seed cultivated land to grass. In recent years, considerable effort has been expended to establish stands of crested wheatgrass (*Agropyron cristatum* (L.) Beauv.). Such attempts have met more often with failure than with success. From 1936 to 1941, for instance, 153,000 acres were seeded to grass under direct supervision of the Soil Conservation Service. Fully half this acreage failed so that reseeding was necessary; in many fields as many as 4 or 5 successive seedings failed, even with approved cultural practices.

It was evident immediately that in many cases, soil on which field plantings of grass had failed would not produce a stand of healthy grass seedlings in the greenhouse, regardless of the moisture supply, and that similar soil, when steamed, yielded excellent stands of vigorous grass seedlings.[2]

There is limited information regarding the nature of forage grass seedling and root troubles in the northern Great Plains. Sprague (4) reported recovery of *Pythium arrhenomanes* Drechsler, *Helminthosporium sativum* P. K. and B., and *Fusarium spp.* from the roots of wheat and a number of forage grasses in the Dakotas. *H. sativum* and *Fusarium spp.* were taken from diseased grass seedling roots collected in eastern South Dakota. Later Sprague and Atkinson (5) reported the susceptibility of 18 species of grasses and cereals to isolates of *P. arrhenomanes* taken from all the species. Recently, Fischer, Sprague, Johnson, and Hardison (3) listed 42 grass species as hosts of *P. arrhenomanes,* 39 species as hosts of *Pythium debaryanum* Hesse, 53 as hosts of *H. sativum,* 36 as hosts of *Rhizoctonia solani* Kuhn, and many species as hosts of several species of *Fusarium.*

Still more recently, Sprague (6, 7) pointed out that *Pythium arrhenomanes* or *P. graminicola* Subr. is the principal cause of seedling blight of grasses during May and June, although seed rotting and interruption of germination can be the result of the activity of *P. debaryanum, P. irregulare* Buis., *Helminthosporium sativum,* and *Fusarium scirpi* var. *acuminatum* (Ell. and Ev.). Sprague's observations are essentially in accord with the information recorded in this paper, which was previously pre-

sented in abstract form (2). The observations of Andrews (1) also are in essential agreement with Sprague's.

In the investigations here recorded, the symptoms of blighted seedlings are described and the evidence for regarding *Pythium graminicola* as the causal agent is presented. The pathogens most commonly recovered from roots of blighted grass seedlings were *P. graminicola, P. debaryanum, Helminthosporium sativum,* and *Fusarium spp.* The gross effects of these pathogens on seedlings in steamed and clean field soil indicate that *P. graminicola* is the most virulent, and in pure culture most nearly reproduces the symptoms evident on seedlings in infested field soil.

SYMPTOMS OF SEEDLING BLIGHT OF CRESTED WHEATGRASS

Two distinct symptom complexes occur after crested wheatgrass seeds have been planted in many field soils; (1) some seeds are rotted during early stages of germination, and (2) many of the seedlings wilt and die or become partially defoliated after they are a week or ten days old. There is only occasional deterioration of seedlings less than a week old.

Examination of swollen but otherwise nongerminated seeds reveals a reduction of all but the glumes to a soft watery mass. Rarely this rot involves radicles and epicotyls during early stages of germination.

A very few young seedlings may "damp-off" before the first leaf is fully elongated and expanded. A very light brown discoloration and general softening of the axis in a region extending usually upward from the scutellar node is evident on such plants. · The tip of the first leaf may lose its green color and quickly become yellow and desiccated. On an occasional plant, the first leaf may break over at the tip of the coleoptile.   ·

The majority of seeds planted in field soil germinate normally and the resulting seedlings appear normal for from 1 to 3 weeks after germination, depending on their rate of growth as determined usually by soil temperature. In the greenhouse, low temperatures may delay growth of the seedlings and also the onset of blight; at higher temperatures seedling growth is more rapid, but the first symptoms usually appear sooner and the final loss of stand is greater. In either case, usually within 2 weeks after completion of germination, the first leaves of infected seedlings begin to wilt (Fig. 5, A). Loss of leaf turgor results in the lack of luster and a consequent lead green color common to wilted leaves. Recovery by individual leaves from this wilted condition seldom occurs, even temporarily. Instead there is gradually increased wilting, followed by loss of green color and finally yellowing and death of the entire leaf.

Sometimes the plant's existence terminates with the death of the first leaf, but frequently the growing point survives to feebly produce a whorl of somewhat dwarfed leaves, the lower ones of which then suffer the same fate. These symptoms suggest failure of water supply to the plant and under stringent moisture conditions might be termed "firing." Actually,

they frequently occur on seedlings in wet soil. Eventually many plants succumb and die, sometimes 6 weeks or more after completion of seed germination.

The roots of afflicted seedlings may exhibit a range of brown discolorations and disintegration. Seedlings that die quickly usually have less than an inch remaining of their primary and seminal roots and these may be brown and softened, particularly at the ends. Plants a month old or older give evidence of having lost roots continuously, with development of new roots on living portions of older ones, commonly above lesions. If a plant lives to tiller, it produces sturdy adventitious roots at the crown which tend to be less disintegrated than the primary and seminal roots. Even so, the watery disintegration of several crowns one-eighth inch in diameter has been observed.

Initial symptoms on the very small primary and seminal roots have not been observed. Wherever infection occurs (apparently near the root tip) the tissues evidently become reddish-brown very quickly. Several attempts to observe pathogens in the cells and tissues or roots of very young seedlings have failed.

While the disease may be much worse in some fields or areas than in others, it apparently does not commonly occur in distinct patches, nor is it restricted to individual plants. Since crested wheatgrass field seedlings are not made under clean culture, but rather under conditions favoring a temporary cover of annual weeds, the mass appearance of an afflicted stand in the field cannot be observed.

Altogether, somewhat more than 400 root fragments of diseased seedlings grown in various field soils in the greenhouse were placed on plain agar (without added nutrients) during the winters of 1940–'41 and 1941–'42. From these were observed to develop: *Pythium graminicola,* 158 times; *Fusarium spp.,* 81 times; *P. debaryanum,* 71 times; *Helminthosporium sativum,* 6 times; and *Rhizoctonia spp.,* twice. All of these fungi except *Rhizoctonia* have been recovered from crested wheatgrass seedlings grown in the field.

GROSS EFFECTS ON CRESTED WHEATGRASS OF SEEDLING PATHOGENS IN PURE CULTURE IN STEAMED SOIL

In order to evaluate and delineate the roles of *Pythium graminicola, P. debaryanum,* and *Helminthosporium sativum* as seedling pathogens of crested wheatgrass, plantings were made in soil infested at various depths below the seed with these 3 pathogens. Also, seedlings of various ages up to 4 weeks were exposed, in steamed soil, to these pathogens. The results of such exposures indicate the pathogenicity of *P. graminicola* at all depths of infestation and to plants of all ages included in the experiments.

In preliminary experiments, in which potato-dextrose-agar cultures of *Pythium graminicola, P. debaryanum, Helminthosporium sativum,* and

Fusarium spp. were placed in contact with the seed in steamed soil, all markedly interrupted germination except the *Fusarium spp.* Some of the latter were slightly pathogenic to seedlings of crested wheatgrass a week or more after germination but the pathogenic isolates in all cases produced direct necrosis, most often near or above the seed, with resultant stiff, lustrous seedlings rather than dull, wilted ones such as commonly occur in field soil. *Fusarium spp.* therefore were not included in further studies.

Because of the relative infrequency of *Rhizoctonia spp.* in isolation plates of greenhouse and field-grown seedlings, these fungi were not included in soil infestation trials.

EFFECTS OF PYTHIUM GRAMINICOLA, P. DEBARYANUM AND HELMINTHOSPORIUM
SATIVUM AT THREE DEPTHS OF INFESTATION

Entire, potato-dextrose-agar, Petri-dish cultures of *Pythium graminicola, P. debaryanum,* and *Helminthosporium sativum* were placed in steamed soil in 4-inch clay pots at three depths as follows: one-eighth inch, 1 inch, and 2 inches below 50 planted crested wheatgrass seeds. Growths of *P. graminicola* and *P. debaryanum* cover a Petri dish of potato-dextrose agar in 48 hours; *H. sativum* requires a week. The cultures used therefore were 2 and 7 days old, so that all were actively growing and about the same size when placed in the steamed soil. The seeds in all cases were covered to a depth of one-half inch with soil. The soil was kept moist, not wet, with tap water.

The experiment was repeated. The first trial included 2 isolates of *Pythium graminicola,* 1 of *P. debaryanum,* 2 of *Helminthosporium sativum,* and 1 check of sterile agar. The second trial included 2 other isolates of *P. graminicola,* the same and 1 other isolate of *P. debaryanum,* the same 2 isolates of *H. sativum,* and a check of sterile agar. Each check and each isolate of the 3 fungi at 3 depths was represented by duplicate pot cultures in each of the trials.

Original stands and numbers of living, dead, and obviously diseased seedlings 1 month after planting were recorded and are presented in table 1. Six weeks after planting, 1 replicate in the first trial and both replicates in the second trial were washed free of soil, the tops and roots separated, air dried, and weighed. These weights are presented in table 2.

There was failure of germination in all pot cultures infested with *Pythium graminicola,* even when infestation was at a depth of 2 inches. Moreover, of the resultant seedlings, only an occasional one was green and vigorous (Fig. 1, A). Those listed as healthy were so listed because there was no evidence above the soil line of direct or indirect necrosis. Furthermore, the profuse wilting and indirect necrosis of seedlings in *P. graminicola*-infested soil was comparable to that in field soil.

Pythium debaryanum failed to induce disease or to kill plants after, seed germination was completed. On the other hand, *P. debaryanum* ob-

viously destroyed many germinating seeds when placed one-eighth or 1 inch below the seed, but not when placed 2 inches below the seed (Fig. 1, B). Alfalfa seeds subsequently planted in these pots failed to germinate or damped-off. It is assumed therefore that *P. debaryanum* was present and active during germination of the crested wheatgrass seed.

In general, *Helminthosporium sativum* interrupted germination less than *Pythium debaryanum* and *P. graminicola*, and not at all when placed 1 inch and 2 inches below the seed (Fig. 1, D). In 1 of 4 cases (trial 2,
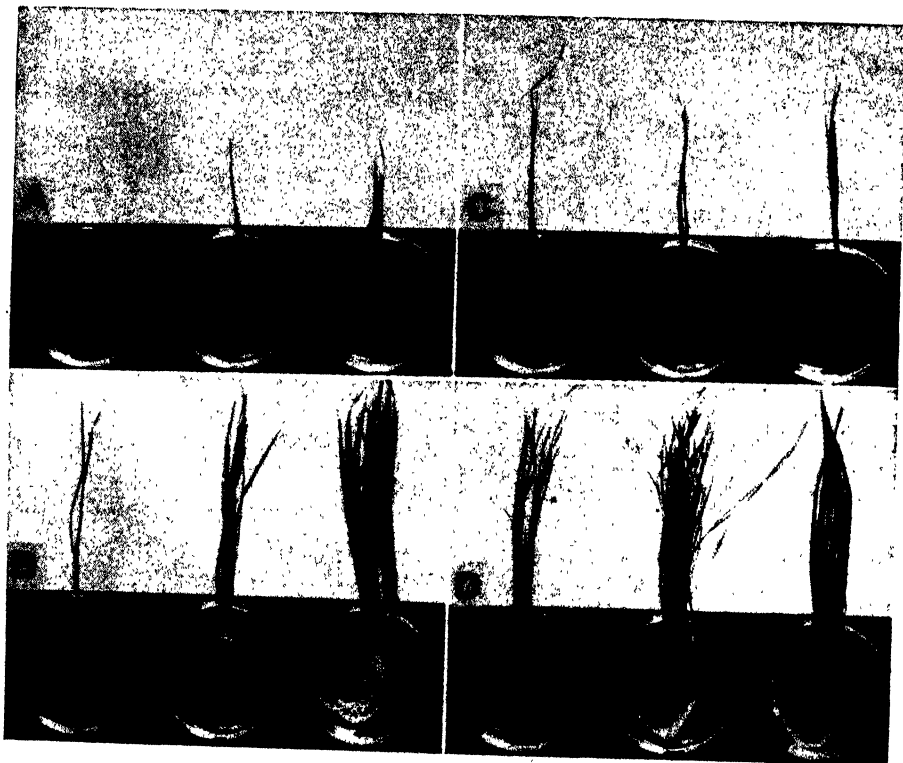

Fig. 1. Crested wheatgrass seedlings grown in steamed soil infested (left to right) one-eighth, one inch, and two inches below the seed with (A) *Pythium graminicola*, (B) *P. debaryanum*, (C) *Helminthosporium sativum*, isolate 12C$_2$, and (D) *H. sativum*, isolate 8C$_2$.

isolate 12 C$_2$) interruption of germination was considerable at all depths (Fig. 1, C). *H. sativum* was more pathogenic to seedlings than *P. debaryanum*, much less so than *P. graminicola*, and like *P. debaryanum*, had no effect when placed at some distance from the seed, especially in trial 1. Furthermore, *H. sativum* induced considerable direct necrosis on turgid, lustrous plants, but very little wilting and indirect necrosis. It should be pointed out that the over-all effects of *H. sativum* isolates 8 C$_2$ and 12 C$_2$ tended to be reversed in trials 1 and 2. No clue to this inconsistency can be offered.

EFFECTS OF PYTHIUM GRAMINICOLA, P. DEBARYANUM, AND HELMINTHOSPORIUM
SATIVUM ON PLANTS OF DIFFERENT AGES

The experiments in which steamed soil was infested at different depths
suggested the possibility that crested wheatgrass seedlings were susceptible

TABLE 1.—*Healthy, diseased, and dead seedlings of crested wheatgrass in steamed
soil infested with Pythium graminicola, P. debaryanum, and Helminthosporium sativum
at depths of one-eighth, 1 inch, and 2 inches below the seed.* (*Average of 2 replicates
of 50 seeds each*)

Fungus species	Depth of infesta-tion be-low seed	Number of seedlings in							
		Trial 1				Trial 2			
		Healthy	Dis-eased	Dead	Total	Healthy	Dis-eased	Dead	Total
	Inches	Isolate 1 C				Isolate 4 C			
Pythium graminicola	⅛	1[a]	1	0.5	2.5	0	1	1.5	2.5
	1	4	4	6	14	2.5	4	12	18.5
	2	6.5	8	9	23.5	8.5	5	13.5	27
		Isolate 3 C				Isolate 8 C			
	⅛	0	0	0 5	0.5	1	0.5	1.5	3
	1	1	2	6	9	4.5	2.5	12	19
	2	2	15	6	23	8.5	8	12.5	29
P. debary anum		Isolate 6 C				Isolate 6 C			
	⅛	5.5	1	0.5	7	2	0	1	3
	1	0.5	0.5	0	1	13.5	0	0	13.5
	2	31	0	0	31	36	0	0	36
		Isolate 6 C				Isolate 7 I			
	⅛	3.5	1	0	4.5	2.5	0	0	2.5
	1	6	0	0	6	34	0	0	34
	2	16.5	0	0	16.5	39	0.5	0	39
Helmintho-sporium sativum		Isolate 8 C₂				Isolate 8 C₂			
	⅛	1.5	1.5	7.5	10.5	15	0.5	5	20.5
	1	13	2	6	21	42	1	0	43
	2	17.5	5	2.5	25[b]	28	4.5	0.5	33
		Isolate 12 C₂				Isolate 12 C₂			
	⅛	0.5	0	8	8.5	2.5	4	5.5	12[b]
	1	25.5	1.5	2 5	29.5	2	2	5	9
	2	33.5	1	0	34.5	0	1	1.5	2.5
Check	⅛	28	0	0	28	41.5	0	0	41.5
	1	29	0	0	29	39.5	0	0	39.5
	2	28	0	0	28	40.5	0.5	0	41

[a] In *Pythium graminicola*-infested soil, "healthy" seedlings were living and not
obviously diseased, but stunted.
[b] Stunted.

at all ages to *Pythium graminicola* but that only nongerminated or germi-
nating seed was susceptible to *P. debaryanum*. The variable results with
Helminthosporium sativum indicated no distinct age-susceptibility pattern.
Since seedlings growing in field soil exhibit symptoms for some time, it is a
sound assumption that the causal agent of seedling blight is pathogenic to
seedlings at least 2 weeks old.

Plants of different ages were exposed to *Pythium graminicola, P. debaryanum,* and *Helminthosporium sativum* in the following manner: Strips of growing Petri-dish cultures of the 3 fungi were laid close alongside rows of crested wheatgrass 5, 8, 12, 15, 22, and 28 days old. The youngest plants, from seed planted 5 days previous, were in reality seeds

TABLE 2.—*Weights, in grams, of dried tops and roots of crested wheatgrass grown in steamed soil, noninfested, and infested with Pythium graminicola, P. debaryanum, and Helminthosporium sativum at depths of one-eighth, 1 inch, and 2 inches below the seed*

Fungus species	Depth of infestation below seed	Weight, in grams, in			
		Trial 1		Trial 2	
		Tops	Roots	Tops	Roots
	Inches	*Isolate 1C*		*Isolate 4C*	
Pythium graminicola	⅛	0	0	0	0
	1	0.04	0.02	0.01	0.01
	2	0.17	0.17	0.03	0.03
		Isolate 3C		*Isolate 8C*	
	⅛	0	0	0.01	0.01
	1	0.01	0.01	0.02	0.02
	2	0.10	0.10	⋅0.04	0.05
		Isolate 6C		*Isolate 6C*	
P. debaryanum	⅛	0.70	0.40	0	0.01
	1	0.06	0.03	0.03	0.03
	2	1.33	1.51	0.36	0.11
		Isolate 6 C		*Isolate 7I*	
	⅛	0.16	0.09	0.04	0.02
	1	0.51	0.45	0.33	0.14
	2	0.94	0.91	0.30	0.12
		Isolate 8C₂		*Isolate 8C₂*	
Helminthosporium sativum	⅛	0.08	0.06	0.01	0.01
	1	0.74	0.47	0.24	0.12
	2	0.20	0.15	0.18	0.09
		Isolate 12C₂		*Isolate 12C₂*	
	⅛	0.17	0.10	0.04	0.02
	1	1.22	1.03	0.04	0.05
	2	1.42	1.15	0.02	0.03
Check	⅛	1.77	1.53	0.30*	0.13
	1	1.26	2.06	0.40	0.13
	2	1.45	1.25	0.35	0.12

*Plants in trial 2 washed 6 weeks after seed planted; in trial 1, 10 weeks after seed planted.

in late stages of germination. The cultures were laid one-half to three-fourths inch below the soil surface about one-half inch to the outside of 1 row in each pot. The other row, about 2 inches distant, was left undisturbed and was intended as a check. Each row consisted of the seedlings from 25 seeds. The experiment was repeated; the first trial consisted of 4 replicates of 1 isolate of each fungus; the second consisted of 2 replicates of each of 2 isolates of each fungus.

After 3 days, a few of the youngest seedlings appeared wilted by each fungus, the most by *Pythium graminicola*, the fewest by *Helminthosporium sativum*. A few young seedlings may have been mechanically injured during the introduction of the cultures. After 5 days, plants of all ages in rows exposed to *P. graminicola* were wilting, particularly the lower leaves (Fig. 2, A), but there were no additional observable pathogenic effects in the rows exposed to *P. debaryanum* (Fig. 3, A) or *H. sativum* (Fig. 4, A).

After 21 days *Pythium graminicola* had killed all the seedlings in the youngest exposed rows and over half the seedlings in exposed rows 3 and 7 days older. Seedlings in exposed rows of all ages were unmistakably wilted

FIG. 2. Crested wheatgrass seedlings exposed to *Pythium graminicola* (at left in each pot) when 5, 8, 12, 15, 22, and 28 days old. Photographed 5 days after exposure (A) and 21 days after exposure (B).

and their lower leaves were undergoing indirect necrosis (Fig. 2, B). Furthermore, all the unexposed rows except perhaps those of the 2 oldest series had wilted and developed symptoms typical of plants infected by *P. graminicola*. Evidently the pathogen had become estabished in the root zone of plants in the rows intended as checks (Fig. 2, B). In the meantime there was no evidence of further development of top symptoms in rows exposed to *P. debaryanum* or *H. sativum* (Fig. 3, B and 4, B).

Ultimately there was partial recovery of some seedlings in all but the youngest rows exposed to *Pythium graminicola*. As a final measure of the effects of these three fungi on seedlings exposed at different ages in this experiment, 1 replicate of the first trial was washed out, air dried, and weighed. The weights of roots and tops were in accord with the appearance
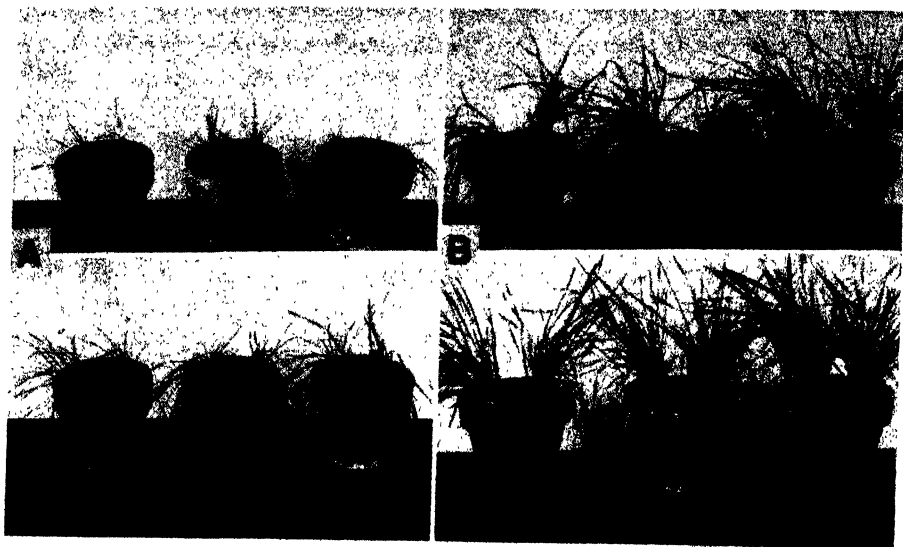
FIG. 3. Crested wheatgrass seedlings exposed to *Pythium debaryanum* (at left in each pot) when 5, 8, 12, 15, 22, and 28 days old. Photographed 5 days after exposure (A) and 21 days after exposure (B).

of the plants; only plants exposed to *P. graminicola* were light in weight, especially those very young when exposed.

It is obvious that *Pythium graminicola* was pathogenic to crested wheatgrass seedlings at least 28 days old, and that *P. debaryanum* and *Helminthosporium sativum* had little or no effect on any but very young seedlings. The results in the second trial were the same as in the first.

FIG. 4. Crested wheatgrass seedlings exposed to *Helminthosporium sativum* (at left in each pot) when 5, 8, 12, 15, 22, and 28 days old. Photographed 5 days after exposure (A) and 21 days after exposure (B).

EFFECTS OF PYTHIUM GRAMINICOLA, P. DEBARYANUM, AND HELMINTHOSPORIUM
SATIVUM ON CRESTED WHEATGRASS SEEDLINGS IN NONSTEAMED
BROMEGRASS SOD

Experiments thus far reported were with steamed soil. The shortcoming of assuming pathogenicity of root-infecting fungi on the basis of infestation tests with steamed soil are obvious and have been demonstrated repeatedly. During the course of other investigations, nonsteamed soil from a plot of bromegrass sod had repeatedly yielded stands of healthy crested wheatgrass seedlings comparable to those in steamed soil.

Pots of this bromegrass sod soil, nonsteamed, were infested with entire Petri-dish cultures of *Pythium graminicola*, *P. debaryanum*, and *Helminthosporium sativum*. The cultures were placed at a depth of one-half inch
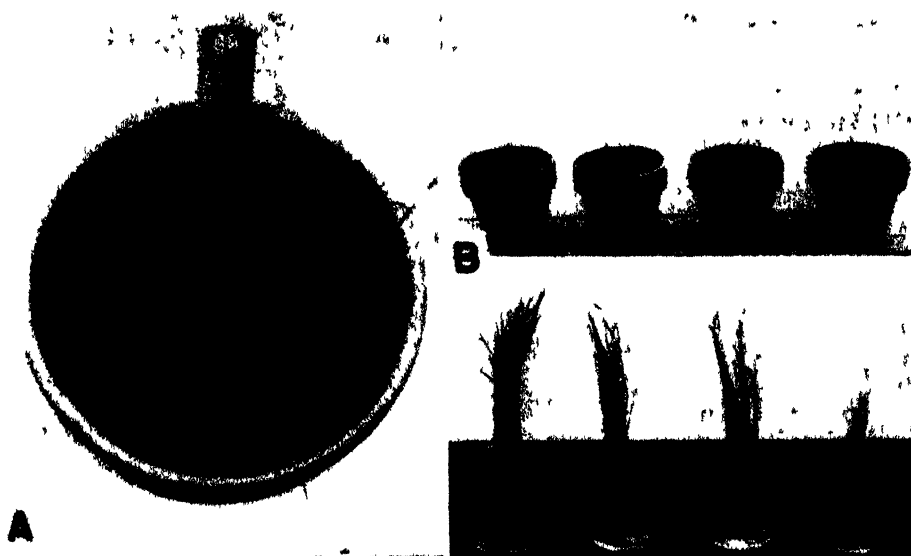

Fig. 5. A. Typical wilting of crested wheatgrass in early stages of seedling blight in infested field soil. B. Crested wheatgrass seedlings grown in non-steamed bromegrass sod. Left to right, sod not infested, infested with *Helminthosporium sativum*, infested with *Pythium debaryanum*, and infested with *P. graminicola*.

below 50 crested wheatgrass seeds planted in each pot. Number of seedlings and air-dry weights of tops and roots are presented in table 3. There was considerable interruption of germination in pot cultures infested with *P. graminicola*, and the seedlings in these pots were wilted and stunted; some were killed (Fig. 5, B). There were no obvious effects from infestations with *P. debaryanum* and *H. sativum*. Results were the same in a second trial. Each trial consisted of 3 replicates of the check and 2 isolates each of *P. graminicola*, *P. debaryanum*, and *H. sativum*.

It is obvious that in these experiments with nonsteamed field soil, *Pythium graminicola* was pathogenic to germinating seed and seedlings of crested wheatgrass and *P. debaryanum* and *Helminthosporium sativum* were not.

RECOVERY OF PYTHIUM GRAMINICOLA, P. DEBARYANUM, AND HELMINTHOSPORIUM
SATIVUM FROM GERMINATING SEED AND YOUNG SEEDLINGS OF CRESTED
WHEATGRASS AT VARIOUS STAGES OF GROWTH

Soil infestation trials revealed that *Pythium graminicola, P. debaryanum,* and *Helminthosporium sativum* can interrupt germination of crested wheatgrass seed, but that only *P. graminicola* is capable of stunting, wilting, and killing seedlings under a variety of circumstances, and particularly in nonsteamed soil. Evidence that *P. graminicola* is the cause of crested wheatgrass seedling blight would seem to be complete, except that the sequence of symptoms is different in field soil than in soil infestation experiments.

TABLE 3.—*Numbers and weights of tops and roots of crested wheatgrass seedlings grown in nonsteamed bromegrass sod infested with Pythium graminicola, P. debaryanum, and Helminthosporium sativum. Averages of 3 replications. Weights in grams on air-dry basis*

Fungus species and isolate	No. of seedlings	Weight, in grams, of	
		Tops	Roots
Pythium graminicola			
Isolate 1	3.3	0.45	.18
Do 2	2.3	0.34	.10
P. debaryanum			
Isolate 1	27.3	2.37	1.51
Do 2	31.0	2.68	1.70
Helminthosporium sativum			
Isolate 1	31.0	2.26	1.56
Do 2	31.6	2.17	1.13
Check	28.6	1.95	.98

It will be recalled that a growing culture of *Pythium graminicola* was pathogenic immediately to germinating crested wheatgrass seed or to seedlings of any age up to at least 28 days. On the other hand, the sequence of symptoms in field soil is: (1) more or less failure of germination, (2) apparently normal development of most seedlings for about a week followed by (3) wilting, stunting, and death of the seedlings or indirect necrosis of the lower leaves.

An attempt was made to reconcile this apparent difference in sequence of symptoms in experimentally-infested steamed soil and naturally-infested field soil by isolating at intervals from seeds germinating and seedlings growing in the latter.

Four soil samples were chosen, 1 each from plots continuously cropped to wheat, peas, sorghum, and alfalfa. The first 2 were known to be heavily infested with both *Pythium debaryanum* and *P. graminicola;* the sorghum plot was likewise infested, but especially heavily with *P. debaryanum.* Soil from the alfalfa plot had repeatedly yielded stands of healthy crested

wheatgrass seedlings comparable to those obtained in steamed soil and was assumed to be free of *P. graminicola*.

These soil samples were distributed, each in a series of pots, with 25 seeds planted in each pot. All the germinating seed or seedlings were removed from 2 pot cultures of each soil sample series on the first, fourth, and eighth day after planting. All the seedlings were removed from 1 pot culture of each series on the eleventh, fifteenth, and twenty-second day after planting. A convenient number of seedlings (6 or 12) was removed from 1 pot culture of each series on the thirty-third day after planting. All germinating seeds and seedlings removed were washed and placed on water agar plates and the resulting growths from them of *Pythium graminicola*, *P. debaryanum*, and *Helminthosporium sativum* were observed and recorded.

The following facts and trends, taken from the results as presented in table 4, are pertinent. Most of the occurrences of *Pythium debaryanum* were in isolations from germinating seed or from seed or seedlings up to 11 days old (15 days after planting). In the soil from the continuous wheat and continuous peas plots, all but 3 occurrences of *P. debaryanum* were in isolations from seedlings up to a week old (11 days after planting). *P. graminicola* appeared only once in isolations from germinating seeds. It developed far more frequently from seedlings 11 days old (15 days after planting) or older. The abundant appearance of *P. graminicola* on isolation plates preceded somewhat, or coincided with, the occurrence of diseased seedlings among those from which the isolations were made. In general, with an increase in age of seedlings there was a tendency for less *P. debaryanum* and more *P. graminicola* to develop in isolations from them. The occurrence of *Helminthosporium sativum* in isolation plates conformed to no particular time pattern in relation to the occurrence of seed rotting or seedling blight.

These relationships are also worthy of note: In other extensive experiments, seedling blight in soil from the plot of continuous peas was as bad as or slightly worse than in soil from the continuous wheat plot. The isolation results indicate the presence of little or no *Helminthosporium sativum* in the soil cropped to peas as compared with the continuous wheat soil. Furthermore, in the same experiments, there was more seed rotting in the continuous sorghum soil than in the continuous wheat soil. Here again, the isolation results indicate less *H. sativum* in the sorghum soil than in the continuous wheat soil. It is evident that considerable seed rotting or considerable seedling blight did occur in field soil with either an abundance or scarcity of *H. sativum* as indicated by isolation results.

In general then, it appears from these isolation results that *Pythium debaryanum* rather than *P. graminicola* is associated with germinating seed of crested wheatgrass in field soil. Conversely, *P. graminicola* rather than *P. debaryanum* is associated with the roots of seedlings with blight symptoms, particularly at the time those symptoms are occurring.

All of these associations are in accordance with the observed potentialities of *Pythium debaryanum* and *P. graminicola* in pure culture, soil infestation experiments, excepting one. The lack of *P. graminicola* in isolations from germinating seed, even in soil in which it later parasitizes the roots of seedlings profusely, can not be accounted for on the basis of the evidence presented. That lack, however, seemingly constitutes a reasonable basis for the normal growth of seedlings for at least a week in heavily infested field soil.

TABLE 4.—*Occurrences of Pythium graminicola, P. debaryanum, and Helminthosporium sativum in isolations at intervals from crested wheatgrass seedlings growing in four non-steamed soils*

| Soil source | Days after planting | Number of isolations | Occurrences of | | | Disease |
			Pythium graminicola	*Pythium debaryanum*	*Helminthosporium sativum*	
	1	43	1	17	1	Present[a]
	4	45	0	6	17	do[a]
Continuous	8	48	5	11	5	Present
wheat	11	22	7	8	2	do
	15	19	10	1	1	do
	22	17	6	0	1	Abundant[b]
	33	12	8	1	0	do[b]
	1	38	0	19	0	Present[a]
	4	47	0	23	1	do[a]
Continuous	8	44	9	8	3	Present
peas	11	21	4	7	2	do
	15	18	17	0	1	Abundant
	22	14	2	1	1	Abundant[b]
	33	12	8	0	1	do[b]
	1	43	0	27	1	Present[a]
	4	45	0	27	0	do[a]
Continuous	8	45	0	20	0	Absent
sorghum	11	22	0	12	0	do
	15	21	0	13	0	do
	22	20	11	0	3	Present[b]
	33	12	2	0	0	do[b]
	1	42	0	7	0	Present[a]
	4	31	0	1	1	do[a]
Continuous	8	41	1	0	1	Absent
alfalfa	11	22	0	7	0	do
	15	22	0	0	0	do
	22	21	0	0	0	do
	33	6	0	0	0	do

[a] Germinating seed diseased.
[b] Seedling blight.

SUMMARY AND CONCLUSIONS

Crested wheatgrass seed, when planted in field soil in which seedling stands fail: 1) may fail to germinate as a result of rotting in early stages of germination; 2) may germinate and give rise to seedlings which develop normally for at least a week, sometimes 2 or 3 weeks; after which occurs 3) wilting, stunting, and indirect necrosis of leaves or entire plants.

An attempt to reproduce this symptom sequence involved these procedures and results:

Isolations from greenhouse-grown seedlings revealed the presence of *Pythium graminicola, Fusarium spp., P. debaryanum,* and *Helminthosporium sativum* in that order of predominance. *Rhizoctonia spp.* were recovered occasionally. All these fungi except *Rhizoctonia* were taken from field-grown crested wheatgrass seedlings.

In infestation experiments with steamed soil, *Pythium graminicola* was pathogenic to germinating seed and seedlings of crested wheatgrass even when placed 2 inches from the seed and was pathogenic to seedlings up to 28 days old. *P. graminicola* induced wilting comparable to that of seedlings in nonsteamed field soil. *P. debaryanum* was pathogenic to germinating seed when placed one-eighth or 1 inch below the seed, but not when placed 2 inches below the seed. *P. debaryanum* possibly was pathogenic to very young seedlings. *P. debaryanum* did not induce typical wilting. *Helminthosporium sativum* varied in its effects, but in general was pathogenic to germinating seed when placed near it and was noticeably pathogenic only to very young seedlings. *H. sativum* did not induce typical wilting.

In nonsteamed soil taken from a plot of bromegrass sod, only *Pythium graminicola* induced typical wilting and stunting of crested wheatgrass seedlings.

In isolations at intervals from germinating seed and seedlings of crested wheatgrass in lots of soil from plots variously infested, there was a tendency for less *Pythium debaryanum* and more *P. graminicola* to appear on isolation plates with increase in time after planting. Abundant occurrence of *P. graminicola* preceded somewhat or coincided with the occurrence of diseased seedlings among those from which isolations were made. The occurrence of *Helminthosporium sativum* in isolation plates conformed to no particular time pattern in relation to the occurrence of seed rotting or seedling blight. There was no definite relationship between incidence of seed rotting or seedling blight and the frequency of occurrence of *H. sativum* on isolation plates.

In these experiments, *Pythium graminicola* clearly duplicated the symptoms of seedling blight and may be considered its causal agent. The absence of *P. graminicola* on germinating seed, plus the abundance there of *P. debaryanum*, with its demonstrated capacity for interrupting germination, leads to the conclusion that *P. debaryanum* is the principal cause of seed rotting. *Helminthosporium sativum* apparently may be a factor in both seed rotting and blighting of seedlings, but a minor, atypical, and inconsistent one.

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TRANSMISSION OF SUGAR BEET YELLOW-NET VIRUS BY THE GREEN PEACH APHID[1]

E D W A R D   S .   S Y L V E S T E R [2]

The yellow-net virus disease of sugar beets has been described previously by the author (14). Briefly the symptoms consist of a bright yellow chlorosis of the veins and veinlets of affected leaves. The vector, the green peach aphid, *Myzus persicae* (Sulzer), has been reported, but details of the relationships between the vector and the virus have not been fully described. This paper presents data relevant to that phase of the work.

MATERIALS AND METHODS

The virus source was originally obtained at Sherman Island, California, and plants inoculated from these original diseased beets were used as virus sources.

The noninfective aphid colonies were maintained upon healthy sugar beet plants in the greenhouse. The colonies were routinely checked for noninfectivity. The aphid transfers were made by the standard camel's-hair brush technique.

The test plants were grown from seed, planted in flats and transplanted to 4-inch pots. The plants and insects were maintained in the greenhouse, and during inoculation they were kept under caged conditions.

DEFINITION OF TERMS

The following terms are used in the paper in describing the relationships between the vector and the virus:

Infection feeding (18) :—The time that a previously noninfective vector is fed on a virus source.

Test feeding (18) :—The time that a vector is fed upon a healthy recipient host, or test plant.

Pre-penetration time:—A variable period of time elapsing between the time an insect is placed upon a plant and the time that penetration begins. This corresponds to the "penetration time" of Watson (16).

Acquisition threshold:—The minimum time necessary for a vector to feed upon a disease source in order to obtain an infective charge of virus.

Inoculation threshold:—The minimum time necessary for an infective vector to feed upon a healthy plant in order to successfully inoculate that plant with a given virus. The term is equivalent to the "threshold" period as used by Storey (12).

Latent period:—The term in common usage which denotes the time that must elapse before a vector can be demonstrated to be infective, after surpassing the acquisition threshold. Two common synonyms for this term are "waiting" and "incubation" period (11).

Retention period:—The period of time, following an infection feeding, during which a vector is capable of producing infections if fed upon a succession of susceptible healthy plants.

Infective:—The term commonly used to describe the condition of a vector which has virus present in its body and is potentially capable of transmitting that virus.

Actively infective:—A condition of an infective vector during which it will successfully inoculate a healthy susceptible host, if given a suitable test feeding upon it.

Passively infective:—A condition of infectivity when a vector will not inoculate a healthy susceptible host, even though given an adequate test feeding period upon that host.

PRE-PENETRATION TIME

Early in the work it was recognized that an aphid, when transferred from one host to another, required a certain period of time to recover from the manual act of transference and begin to feed upon the new host plant.

Watson (16) used the term "penetration time" in referring to this phenomenon and reported an average of 4.88 minutes for the completion of the period.

Since aphids, during this period, are not penetrating any tissue in a permanent sense, it is felt that the term "pre-penetration" time might be used with more accuracy to denote the presence of this period. An aphid, during this time, may make trial penetrations, but these are usually but a few seconds in duration. For convenience, in this particular work, these are not considered true penetrations, which usually are relatively permanent and terminate in the phloem tissues of the plant (10, 15).

The importance of the pre-penetration period is realized when attempting to determine the inoculation or acquisition threshold.

Based upon data taken upon 64 watched individuals, the minimum pre-penetration period noted was 30 seconds, while the maximum was 13 minutes, 9 seconds. Individuals which wandered above this maximum were discarded as being abnormal, either inherently, or due to injury during transference. The average pre-penetration time was 4.9 minutes.

ACQUISITION THRESHOLD

The acquisition threshold, as used in this paper, is applied to the shortest time determined that a previously noninfective aphid must feed upon a disease source, before it is able to acquire an infective charge of the virus. The pre-penetration time was allowed to elapse before the timing of the infection feeding period was begun.

To determine this threshold, a single previously noninfective mature aptera of the green peach aphid was placed upon a virus source plant and allowed to complete the pre-penetration period. It then was given an infection feeding period of definite length according to the experiment. The intervals used were 2, 4, 5, 10, 15, 20, and 30 minutes infection feeding upon the diseased source. The results of the trials are shown in table 1.

The results indicate that apterae are capable of acquiring sufficient virus to become infective within 5 minutes after they have placed the rostrum in position for penetration.

Penetrated tissue studies by Roberts (6) indicated that only 11 per cent of the stylet tracts of green peach apterae will attain the phloem of sugar beet leaves in 15 minutes.

Since an acquisition threshold was obtained in a time too short to allow the aphids to reach the phloem tissues, it would appear that the virus is present in tissues of the mesophyll. These results obtained are somewhat comparable to those of Watson (17) in experimental transmission of the beet-yellows virus with the green peach aphid.

TABLE 1.—*Results* of trials to determine the acquisition threshold of the yellow-net virus, using single previously noninfective green peach aphids, a variable infection feeding period, and a 24-hour test feeding period

| | Infection feeding in minutes | | | | | | |
2	4	5	10	15	20	30	Total
0/5	0/5	4/40	1/75	2/60	0/5	1/20	8/210

ᵃ In the ratios listed, the numerator indicates the number of plants that were positive, while the denominator shows the number of plants inoculated.

INOCULATION THRESHOLD

The inoculation threshold, as defined, is the minimum time necessary for an infective vector to feed upon a healthy plant in order to successfully inoculate that plant with a given virus.

The experimental procedure used to determine this threshold was to allow a single infective aptera of the green peach aphid to feed, following the completion of the pre-penetration period, upon a healthy beet seedling for a given time. The test feeding period was varied according to the specific experiment. In the preliminary work the aphids, after feeding for the specified time on the healthy beet, were transferred to a second healthy beet and allowed to feed for 24 hours. This served as a partial check upon the vector infectivity. The results are given in table 2.

From the data in the table it would appear as if the inoculation threshold is somewhere near 15 minutes.

Watson (17) found, upon 2 occasions, using a total of 25 plants, that the green peach aphid was able to inoculate a plant with the beet-yellows virus in a test feeding period of 7 minutes. This could not be done with the yellow-net virus.

TABLE 2.—*Results*[a] *of trials to determine the inoculation threshold of the yellow-net virus, using single infective green peach apterae and a variable test feeding period*

| | | | Test feeding in minutes | | | | | |
	2	5	10	15	20	25	30	Total
Test plant	0/5	0/15	0/15	2/30	1/30	1/30	3/10	7/135
Check plant[b]		5/15	6/15	2/10				13/40

[a] In the ratios listed the numerator indicates the number of plants that were positive, while the denominator shows the number of plants inoculated.
[b] The check plant refers to the second beet inoculated by the aphid after completion of the test feeding period upon the first recipient host. The feeding period on the check plant was at least 24 hours.

The results indicate that in order for an infective insect to inoculate a healthy beet seedling, the feeding period must be at least 15 minutes or approximately the period of time required by the insect to reach the phloem region of a beet seedling. Apparently the insect must place the virus in, or near, the phloem region if inoculation is to be achieved.

Determination of the inoculation threshold aids in the interpretation of latent period data, and possibly in ascertaining which tissue region of a plant must be attained by a vector if successful inoculation with a virus is to occur.

LATENT PERIOD

The time necessary for a vector to become infective after having access to a virus has been variously termed as "waiting" or "incubation" period as well as the "latent" period (7, 11). The writer prefers the term "latent period" and would use "incubation period" for designating the time necessary for symptoms of disease to develop within a plant.

The method employed to determine the latent period was as follows. Previously noninfective insects were placed upon a virus source for a given length of time. The length of the infection feeding period was varied from 15 minutes to an hour. At the end of this feeding period the insects were transferred singly to a healthy recipient host and allowed to feed for a given test feeding period. This period was varied from 15 minutes to 45 minutes. At the termination of the test feeding period the vector was removed and the plant fumigated. The results of the trials are given in table 3.

TABLE 3.—*Results*[a] *of trials to determine the latent period of the yellow-net virus within the green peach aphid, using previously noninfective single apterae, a variable infection feeding period, and a variable test feeding period*

| | | | Time in minutes | | | | |
| Infection feeding | 15 | 30 | 15 | 30 | 30 | 60 | Total |
Test feeding	15	15	30	30	45	30	
	0/5	0/5	1/5	1/5	0/5	1/5	3/30

[a] In the ratios listed the numerator indicates the number of plants that were positive, while the denominator shows the number of plants inoculated.

The shortest total time period which yielded positive results was 45 minutes. Since the acquisition threshold has been determined to be approximately 5 minutes, and the inoculation threshold approximately 15 minutes, in the total time example of 45 minutes there remained a possible latent period of 25 minutes. This 25-minute interval could be a true latent period, or only an apparent one, depending upon whether it is assumed that one, or the other, or both, of these thresholds was prolonged in the case of the particular trial.

The results are somewhat similar to those obtained by Watson (17), using the green peach aphid as the vector for the sugar beet-yellows virus, which led her to conclude that there was no sharply defined latent period. The same conclusion appears to be justified in the case of the yellow-net virus.

VECTOR EFFICIENCY

Vector efficiency can be defined as the transmission expectancy, expressed as percentage, when a vector is given one access period to a diseased source and subsequently transferred to the first healthy recipient host.

During retention studies it was learned that a given insect often would fail to transmit the virus, as evidenced by the appearance of symptoms, to the first of a series of healthy recipient beets. This will directly affect the vector efficiency results. Therefore, it is felt that the use of the term efficiency, for convenience and for purposes of this paper, should be limited to the results of one transfer of an insect from a virus source to a healthy recipient host. The time spent in the infection feeding and test feeding periods should be long enough to allow a near maximum of infectivity and transmission to occur. At best the vector efficiency rating, under the restrictions of the definition, will give only a partial estimate of the number of insects actually infective.

The problem of vector efficiency was approached by combining data from numerous experiments and the efficiency of a "vector-group" composed of 10 apterae, was compared with the efficiency of single apterae. In the examples used, the infection feeding period was 24 hours, and the test feeding period was 24 hours. Based upon 207 examples, it was found that a vector-group can be expected to transmit the yellow-net virus approximately 75 per cent of the time under the limitations of the definition. The vector efficiency rating for a single aptera, based upon 125 examples, is approximately 26 per cent.

VIRUS RETENTION

Early in the experimental work it was learned that the virus was of the "persistent" type, as defined by Watson and Roberts (19), i.e., the vector, after once becoming infective retains the ability to transmit the virus for a relatively long period of time.

This does not imply that an infective insect will infect every plant that it is fed upon, but rather that when an insect is transferred to a series of healthy plants until its death, some positive plants will appear in the series.

TABLE 4.—*Results* of trials to determine the ability of vector-groups, composed of 10 infective green peach aphid apterae, to retain the yellow-net virus. The aphid groups were transferred daily to successive healthy beet seedlings

Aphid group No.	Day transferred										
	1	2	3	4	5	6	7	8	9	10	11
1	+	+	+	+	+	+	+	+	+	+	+×
2	+	+	+	+	+	+	+	+	+	−	−×
3	+	+	+	+	+	+	+	+	−	−×	
4	+	+	+	+	+	+	+	+	+×		
5	+	+	+	+	+	+	−	+×			
6	0	+	+	+	+	+	−	+	−	−×	
7	+	+	+	+	+	+	+	+	−×		
8	+	+	+	+	+	+	−×				
9	+	+	+	+	+	+	+	+	−×		
10	−	+	0	+	+	−	+×				

ᵃ The plus sign (+) indicates the production of the disease, and the minus sign (−) shows that no disease resulted. The zero sign (0) indicates that the plant died, while the (×) sign indicates the day that all insects were dead, and transfers were discontinued.

Tables 4 and 5 give in detail the results of retention experiments, using both vector-groups and single insects. Table 5 indicates the irregularity of the intervals with which the infection-producing feedings occur when manipulating single insects, whereas in table 4, this irregularity appears in the vector-group only when it has been reduced by mortality to approximately a single individual.

EFFECT OF MULTIPLE FEEDING PUNCTURES UPON VIRUS TRANSMISSION

The appearance of negative plants in series of daily transfers of infective insects to healthy plants has been noted many times. In fact, Freitag (4)

TABLE 5.—*Results* of trials to determine the ability of single infective green peach aphid apterae to retain the yellow-net virus. The single apterae were transferred daily to successive healthy beet seedlings. Sixteen of the 30 aphids tested failed to transmit the virus in any of the transfers and are not included in the table

Single aphid No.	Day transferred						
	1	2	3	4	5	6	7
1	+	−	−	−	−×		
3	+	−	−	−×			
4	−	−	+	+	−×		
7	−	−	−	+×			
9	−	−	−	+×			
10	−	−	−	−	−	+	−×
18	−	−	+	−	−	−×	
21	+	+	−×				
22	+	−	+	+	+	+×	
24	+	+	+×				ᵞ
25	+	+	−×				
28	−	+×					
29	+	+	−	+	+×		
30	−	−	−	+×			

ᵃ The plus sign (+) indicates the production of the disease, and the minus sign (−) shows that no disease resulted. The (×) sign indicates the day that the insect was dead and transfers were discontinued.

and later Bennett and Wallace (1) used this phenomenon in conjunction with other evidence, as indicating the lack of multiplication of the curly-top virus within its vector, *Eutettix tenellus* (Baker). The explanation of the occurrence of the negative plants will vary according to the initial assumption that is made as to the mechanics of virus transmission by insects.

Two assumptions are possible. One, accepted by Storey (13), is that an infective insect has periods when it is "actively infective", *i.e.*, periods during which it will eject virus during feeding, alternating with periods during which it is "passively infective", *i.e.*, periods during which it does not eject virus, even though it is feeding. Also, the passively infective periods increase in number and duration as the length of time since the last infection feeding increases. This implies that virus ejection by an infective insect is irregular during the course of its feeding, and that the explanation for the "skips" in transmission is that the insect was passively infective, *i.e.*, failed to inject virus, during a particular test feeding.

The second assumption as to the method of virus ejection is that the virus, as long as it is present in the insect, is being ejected continuously as the insect feeds. In this case the reasons for the "skips" in a retention series would be (1) plant resistance, (2) ejection of virus into tissue unfavorable for multiplication or translocation, (3) the injection of a subminimal infective dose of the virus, or (4) any combination of the above mentioned factors.

The influence of the factor of plant resistance has been shown to be operating in certain cases by Giddings (5), and thus in some cases it can be the primary factor operating against transmission. But even in the case of a very resistant host, if any percentage of transmission was established, a typical pattern exhibited in a retention series undoubtedly could be identified which would not be explicable upon the basis of plant resistance.

The importance of the factor of a favorable tissue region for successful virus invasion might be difficult to demonstrate experimentally. Sheffield (9) has shown that individual cells of the same tissue appear to vary in their susceptibility to a given virus. Consequently the existence of such a factor must be borne in mind.

The third factor, that of injection of a subminimal infective dose of virus, is the one upon which the idea of mass inoculation, as interpreted by Carsner and Lackey (2) and Severin (8), depends for its experimental proof. The factor possibly acts in certain cases, but the consistent failure to obtain conclusive experimental data appears to relegate it to a position of minor importance in the consideration of reasons for the appearance of negative plants in a retention series.

In order to test the hypothesis of continuous virus injection during feeding, the following experiment was done, with two variations. A colony of green peach aphids was established on a virus source plant. When the colony was of sufficient size, aphids were removed as pairs, *i.e.*, two aphids

which were feeding on the same vein, or veinlet, or which were feeding as closely together as possible, were selected and each was put on a separate healthy beet seedling. One of the aphids of the "pair" was allowed to feed continuously for the entire test feeding period. The other member of the pair was disturbed at 30-minute intervals so as to force it to make at least 10 punctures.

In one experiment the total test feeding period was approximately 24 hours, and the disturbed aphid of the pair, following the 4-hour discontinuous feeding was allowed to remain feeding upon the same plant until the termination of the test feeding period, or approximately 20 hours. In this experiment a total of 20 aphid pairs was used. The continuously fed aphids successfully inoculated 6 out of 20 plants fed upon, while the discontinuously fed aphids successfully inoculated 8 out of 20 plants fed upon.

In the second experiment, each pair was allowed a total test feeding period of 7 hours, and the disturbed member was forced to move at 30-minute intervals for the first one and one-half hours, at one and one-half hour intervals for the next 3 hours, and at 30-minute intervals for the next two and one-half hours. At the end of the final half hour interval all the plants in the experiment were fumigated. In this experiment a total of 50 aphid pairs was used. The results showed that the continuously fed aphids successfully inoculated 17 out of the 50 plants used, while the discontinuously fed aphids successfully inoculated 13 out of the 50 plants used.

The object of the trials was to determine if the amount of saliva that the insect injects into the plant while making the puncture would affect the amount of effective virus ejected. Also an attempt was made to learn if the factor of tissue region penetrated was operating to any degree of effectiveness against transmission.

Work by Davidson (3) has indicated that usually an aphid, when undisturbed for a period of time, will make but one penetration. It was believed, therefore, that by the above experimental procedure a comparison could be made between the effect of a single puncture and its accompanying salivary secretion and tissue region penetrated, and the effect of 10 punctures and their accompanying salivary secretions and 10 different tissue locations. This comparison was to be made upon similar beet seedlings with two vectors which had fed upon the same virus source, and as nearly as possible, in the same general tissue region on that disease source.

It was believed that the inoculation threshold was one of several influencing factors in the experiments not adequately accounted for, and that it might have influenced the results. Since this threshold was determined to be approximately 15 minutes, it was believed that the disturbing intervals might have been of insufficient length to allow proper completion of the inoculation thresholds in all instances. As a result, another experiment was designed in which the interval between the disturbances was lengthened to 1 hour, to allow an adequate inoculation threshold. In addition, one other variant was introduced, in which a third series of aphids was transferred to a new plant every hour.

All the aphids for the experiment were taken from a colony which had been derived from a single green peach aphid aptera. The aphids were fed upon a single diseased source plant, and then transferred to healthy beets in groups of three, *i.e.*, three aphids feeding upon a common vein, or in close proximity to each other were selected and then placed singly upon three healthy beet seedlings. One of the aphids was left undisturbed for 10 hours (continuous feeding). The second aphid was forced to move, by

TABLE 6.—*Results[a] of trials to determine the effect of multiple feeding punctures upon the efficiency of transmission of the yellow-net virus by infective green peach aphid groups, composed of three aphids, fed upon the same virus source. One aphid of each group was allowed to feed continuously for 10 hours, one was disturbed in its feeding at the end of each hour of feeding, until 10 punctures had been made, while the third aphid was transferred to a new beet seedling at the end of each hour, until it had fed upon 10 plants*

Aphid group No.	Continuous feeding	Discontinuous feeding	Hourly serial transfer plant No.										Total[b]
			1	2	3	4	5	6	7	8	9	10	
1	+	+	−	−	−	−	−	−	−	−	−	−	0
2	+	+	+	+	−	−	−	−	−	+	−	−	3
3	+	+	−	−	−	−	−	+	−	+	+	+	4
4	+	−	−	−	−	−	−	−	0	−	−	−	0
5	+	+	−	−	−	−	−	−	−	+	−	−	1
6	+	−	−	−	−	−	−	−	+	−	−	−	1
7	−	−	−	−	−	−	−	+	−	−	+	+	3
8	−	−	−	+	−	−	−	+	−	−	−	−	2
9	−	+	−	−	−	−	+	−	−	+	−	−	2
10	−	−	−	−	−	−	−	+	−	−	−	−	1
11	−	+	−	−	−	−	−	−	−	−	+	−	1
12	+	+	−	−	−	−	−	−	−	−	−	−	0
13	−	+	+	−	−	+	+	−	−	−	−	+	4
14	−	−	−	+	−	−	−	−	−	−	−	−	1
15	+	+	−	−	−	−	+	−	−	−	−	−	1
16	−	−	+	+	−	−	−	−	−	−	−	−	2
17	−	+	−	−	−	+	+	−	+	+	+	−	5
18	+	+	+	−	−	−	−	−	−	−	−	−	1
19	−	+	−	−	−	−	+	−	−	−	+	−	2
20	−	+	0	−	−	−	−	+	−	−	−	−	1
21	−	+	−	+	+	−	−	+	−	−	−	−	3
22	−	+	0	−	−	−	−	−	−	−	−	−	0
23	−	−	+	−	+	−	−	+	−	−	−	−	3
24	−	+	+	+	+	−	−	−	−	+	+	−	5
25	−	+	−	+	−	−	−	−	−	−	−	−	1
Total	9	17	6	7	3	2	5	7	2	6	6	3	47

[a] The plus sign (+) indicates the production of the disease and the minus sign (−) shows that no disease resulted. The zero sign (0) indicates that the plant died.

[b] This total represents the total number of plants infected by each aphid in the serial transfers, out of a possible 10 fed upon.

probing with a camel's hair brush, at the end of each hour until it had made 10 moves, and theoretically 10 punctures (discontinuous feeding). The third aphid of the group was transferred to a new beet seedling at the end of each hour until it had fed upon 10 plants (serial feeding). This procedure was repeated 25 times. The seedling beets used in the experiment were either in the cotyledon stage or had one true leaf emerging. The plants were fumigated with nicotine at the termination of the experiment,

and were placed upon one bench in the greenhouse for incubation. The data obtained are in table 6. The results indicate that increasing the number of punctures made by a single infective insect upon a single plant has a beneficial effect upon the transmission of the yellow-net virus. This increase in vector efficiency has statistical support ($x^2 = 5.12$ for 1 degree of freedom). It also is evident that the effective salivary ejection, as far as the virus transmission is concerned, must be initial, since the total time spent by the two groups in the test feeding period was the same, *i.e.*, virus transfer occurs during penetration or shortly after the puncture is terminated. Actually the effective feeding time was much shorter in the discontinuous feeding trials, because of the influence of the inoculation threshold, and yet the increase in infection percentage was found in these trials.

The actual increase in the number of infections obtained in the discontinuous trials could have been due to (1) overcoming a plant resistance factor, by the introduction of more virus-bearing saliva as a result of multiple punctures, by attaining a favorable tissue region, etc., or it could have been due to (2) discontinuous effective virus contamination of the salivary secretion, *i.e.*, the insects had both passively and actively infective phases during the test feeding periods, and the procedure accentuated the effect of the actively infective phases.

In the second phase of the experiment an attempt was made (1) to determine the efficiency of the insects by again forcing them to feed 10 times, and also (2) to reduce the effectiveness of a possible factor of plant resistance by allowing the aphids a choice of 10 plants. If the effective salivary ejection occurred during the initial stage of penetration, the demonstrable percentage of infective insects would have been as it was in the discontinuous series. This was true, with a slight, but not significant ($x^2 = 1.74$ for 1 degree of freedom), increase in the serial trials.

This increase might have been due to the operation of a plant resistance factor, but if this was the case, its significance was slight. In the serial transfer trials, negative series of plants occurred in 4 cases, while 8 occurred in the discontinuous trials, and it would seem improbable that this would have been due to plant resistance. However, it readily could be explained by assuming the insect was actually noninfective or was in a long passively infective state.

The same reasoning can be applied to the nine examples in which only one infection out of a possible 10 occurred in the serial transfers. Here again it would appear as if the series of skips was due to the insects being passively infective for a series of plants, then actively infective, and again passively infective. This concept of the manner in which the aphids transmit the yellow-net virus would appear to be the more probable explanation of the results obtained in the experiment.

These data, along with those obtained in retention trials, seem to support the opinion that the actual injection of virus into the plant via the salivary secretion is irregular, and that as the infective insect grows older, the periods

of time during which the insect is passively infective are lengthened considerably (4).

TRANSMISSION BY MORPHOLOGICAL VARIANTS

It was decided to determine whether or not the various morphological forms of the aphid species would transmit the virus, and whether or not the power of an individual to transmit the virus would be retained through a moulting period. A colony of green peach aphids was established on a diseased source plant and allowed to increase until "pupae" (young nymphs possessing wing pads which indicate that they will develop into winged, or alate, forms) were present along with the apterae. Pupae from the disease source were placed singly upon healthy beet seedlings, and

TABLE 7.—Results[a] of trials to determine whether or not the yellow-net virus could be retained by an individual aphid through a moulting period. Single infective green peach aphid pupae were transferred daily to healthy beet seedlings until they died. If a nymph moulted during the course of transference, the resulting alate was transferred daily until its death. The apterae, fed the first day only, served as a check upon the virus source

Pupa No.	Day transferred									Aptera No.	Day transferred 1
	1	2	3	4	5	6	7	8	9		
1	−	−	+	−	−/	−	−×			1	+
2	−	+	+×							2	−
3	+	−	−×							3	−
4	−	−/	−×							4	+
5	−	−	−×							5	−
6	−/	−	−	−	−	−×				6	+
7	−/	−	+	−	−×					7	−
8	−	+	−	−/	−×					8	−
9	−/	−	−	−	−	−	+×			9	−
10	−	−	−	−	−	+/	−	−	+×	10	−
11	−×									11	−

[a] The plus sign (+) indicates the production of the disease, and the minus sign (−) shows that no disease resulted. The diagonal line (/) indicates the day on which the pupa moulted producing the alate, and the (×) sign shows the day on which the insect was found dead.

were subsequently transferred daily until death to healthy seedlings. A comparable number of apterae from the same colony were set up singly on the first day to serve as a check upon the virus source. During the course of the serial transfers some of the pupae moulted and produced the alate form. These resulting alatae were transferred daily until death of the individual occurred. The results of the trials are given in table 7.

Five of the pupae and 2 of the resulting alatae transmitted the virus, and in one case the virus was transmitted by a pupa and the alate resulting from that pupa. This would indicate that the virus can be retained by the vector through a moulting period, and that the two main morphological variations of the viviparous form are capable of transmitting the yellow-net virus.

Retention studies have shown that mature apterae will transmit the virus until the time of their death. An experiment was made to determine the acquisition and transmission of the virus by young nymphs. A mature aptera placed on a virus source plant for 24 hours was transferred together with one of its young to individual healthy beet seedlings. Twice in 16 replications was the virus transmitted by the first instar nymphs, thus completing the knowledge of the range of infectivity to include all common stages of the insect.

TRIALS WITH OTHER SPECIES AS VECTORS

The following species of aphids were tested to determine whether or not they would act as vectors of the yellow-net virus: *Aphis rumicis* Linnaeus, *Macrosiphum solanifolii* (Ashmead) (= *gei* (Koch)), *Myzus circumflexus* (Buckton) and *M. pseudosolani* Theobald (= *convolvuli* (Kalt.), = *solani* (Kalt.) of authors). The results of the trials are given in table 8.

TABLE 8.—*Results of trials to determine whether or not several species of aphids will act as vectors of the yellow-net virus. A minimum of 4 and a maximum of 10 individuals were used in each test*

Aphid species	Number of plants inoculated	Number of plants infected
Aphis rumicis Linnaeus	42	1
Macrosiphum solanifolii (Ashm.)	35	3
Myzus circumflexus (Buckton)	25	0
M. pseudosolani Theobald	28	1

A minimum of 4 and a maximum of 10 insects was used in each trial, and all forms, apterae, nymphs, and alatae, were included in the tests. The results indicate that three other species of aphids, viz., *Aphis rumicis* Linn., *Macrosiphum solanifolii* (*Ashm.*) and *Myzus pseudosolani* Theob., aside from the green peach aphid, can serve as vectors of the yellow-net virus of sugar beets under experimental conditions.

In the field the green peach aphid far surpasses any other aphid species in prevalence on sugar beets. *Aphis rumicis* Linn. can be collected in the field on sugar beets, but infestations of this species are localized and irregular.

DISCUSSION

The experimental results which have been obtained during the work have indicated the operation of some of the factors which influence the transmission of the yellow-net virus of sugar beets by the green peach aphid.

The yellow-net virus has been classified as a persistent aphid-borne virus. The terms persistent and non-persistent were introduced by Watson and Roberts (19). Persistent indicates that the virus is retained within the vector for a relatively long time, while non-persistent indicates a relatively short period of retention. Later Watson (18) expressed doubt as to the

value of the degree of retention as the best criterion of separation of the two types of viruses, believing that the influence of a pre-infection feeding fast upon resultant vector efficiency was a more valid measure of distinction. The fast increases the vector efficiency in the case of the non-persistent virus, but does not in the case of the persistent virus. If this latter phenomenon alone is used for separation, then the use of the two terms has lost the word association value, a feature important to the definition of the terms.

One other point needs consideration when using the influence of a pre-infection feeding starvation period upon the vector efficiency as a criterion for persistence or non-persistence. In the case of the non-persistent aphid-borne viruses, the starvation effect is pronounced and can be readily demonstrated. In the case of the persistent aphid-borne viruses, it is doubtful that the factor can be measured under comparable conditions, because of the influence of the relatively long acquisition (5 min.) and inoculation (15 min.) thresholds, which would tend to mask, if not eliminate, this ephemeral phenomenon. The factor has possibilities of being tested in the case of some of the leafhopper transmitted viruses, but again this group of viruses may be unrelated to the aphid-borne persistent viruses.

If it can be considered that the period of retention is relative, and other factors are constant, then there appears to be but little difficulty in classifying a virus either as persistent or non-persistent. It makes little difference whether a virus is retained for 48 hr., 72 hr., or for the functional life of the insect, as long as there exists a number of viruses which will fit into the category of persistent aphid-borne viruses. Likewise it matters little if a virus is retained 15 minutes, or for 6 hours, it still could be considered as a non-persistent virus, relatively speaking, and as long as there is a number of examples of viruses which can be placed into the category, the term serves its purpose. There will be viruses which will be in the transition zone, as to persistence in the vector, and therefore upon this factor alone they could not be classed either as persistent or non-persistent. However, there appear to be 3 factors which would aid in determining the classification of a virus: (1) the period of retention, (2) the effect of a pre-fasting period prior to the infection feeding upon subsequent transmission efficiency, and (3) the status of juice transmissibility of the virus. It follows that a "typical" aphid-borne persistent virus would have the following characteristics: (1) it would be retained by the aphid vector for a relatively long period of time, (2) the vector efficiency would not be influenced by a pre-fasting period prior to infection feeding, and (3) it would not be juice transmissible with ordinary facility. A "typical" non-persistent virus would be opposite in these respects. For convenience, it would seem feasible to classify a virus according to present knowledge as being persistent or non-persistent if it had any combination of 2 or more of the 3 characters in common. If such a system proved to be impracticable in the future, then it should be discarded as have been numerous systems of classification in the past.

The valid use of the term latent period in connection with the persistent aphid-borne viruses is of doubtful value. It appears that the term may be justifiable in the transmission concept of certain of the leafhopper transmitted viruses, and possibly also in the case of one or two of the aphid-borne viruses. However, there are now two examples of persistent aphid-borne viruses, *viz.*, the beet-yellows virus (18) and the yellow-net virus which have been relatively intensively studied and which apparently can be transmitted by the vector without the necessary interpolation of a latent period between an infection feeding and a test feeding when previously noninfective insects are used. From this it would seem that the phenomenon of the latent period has less significance than it formerly was judged to have had in being characteristic of the persistent aphid-borne viruses.

It has been assumed in the past that the saliva injected into a plant by the feeding insect is the vehicle for virus transmission by suctorial insects, and it would seem as if some experimental evidence has been presented which would increase the validity of this established view. When an aphid penetrates plant tissues there is a salivary sheath formed, and at the termination of the puncture some probing action occurs until a suitable location is found for the uptake of food. Using this as a basis, it would appear reasonable that if it were possible to force an infective aphid to introduce 10 times as much salivary secretion into a plant as it normally would in a given period of time, there would be a decided increase in the number of infections obtained, if the saliva was transporting the virus. When this was attempted, an increase in the number of infections was obtained. The other factors which could account for such an increase appear to have been reduced to a minimum by the experimental procedure. Thus it would appear that saliva was the vehicle for virus transport.

The next consideration is whether or not the saliva is ejected continuously during feeding, and therefore whether the insect is continuously injecting virus during prolonged feeding periods. It has been common observation that, within limits, prolongation of the test feeding period increases the probability of successful inoculation. The evidence obtained during these studies indicates that the ejection of effective saliva is initial, *i.e.*, during penetration, or shortly after the puncture is terminated. If such is the case, the reason for the beneficial effect in the prolonged feeding periods would be the greater opportunity for a series of punctures. Likewise, the explanation for a positive correlation between the length of the test feeding period and the increase in vector efficiency, until a maximum is reached, is that finally all virus-potent vectors have established infection and consequently prolongation of the test feeding period would be of no avail.

This would seem to reduce the effect of one continuous feeding period by a vector to a minimum, as far as vector efficiency is concerned.

Whether or not the saliva is always contaminated with virus is also a question. The results obtained show that an insect may be spasmodically

active in producing infections, indicating the ejection at times of non-contaminated saliva · This phenomenon has been described in terms of active or passive infectivity. There appears to be no phasic or cyclic occurrence of the active and passive states of infectivity, but the status for want of a better explanation would seem to be determined by individual "potency", a term introduced by Storey (12).

The practical application of such information possibly would be in the explanation of some of the phenomena observed in connection with field spread of virus diseases. There might exist in the field a large, or small, restless infective population, either because of conditions of crowding, in the case of a very large population, or irritation, in the case of a small population, such as might occur during and following the application of insecticides or fungicides, or cultural practices, when the individual would be making large numbers of punctures, but punctures of relatively short duration. In such a case, rapid virus spread might be expected. Also, if such conditions existed, a rate of virus spread might be found which seemingly would be inconsistent with the population density. On the other hand, it is conceivable that a situation could exist where a large but relatively sedentary population existed in the field, thus there would be a relatively small number of penetrations. In such a case the rate of virus spread might be relatively slow in comparison to the population density.

SUMMARY AND CONCLUSIONS

The vector-virus relationships upon which experimental work was done are described. The primary vector of the yellow-net virus of sugar beets appears to be the green peach aphid, *Myzus persicae* (Sulzer), although evidence indicates that *Aphis rumicis* Linn., *Macrosiphum solanifolii* (Ashm.), and *Myzus pseudosolani* Theob., can act as vectors experimentally.

The acquisition threshold was determined to occur at 5 minutes, while the inoculation threshold is approximately 15 minutes after feeding commences, which would seem to indicate that mesophyll penetration is sufficient for acquisition of virus, but the phloem is the essential tissue involved in inoculation. A distinct latent period does not appear to exist.

The green peach aphid retained the virus for 11 days, or for the duration of its life under the experimental conditions. Tests on the effect of multiple feeding punctures indicated that the saliva introduced into the plant in connection with the puncture, viz., the salivary sheath, or the saliva ejected shortly thereafter, is the vehicle used for virus transport. Data presented indicate that virus contamination of the saliva is not continuous, but rather discontinuous. To describe the phases of vector infectivity resulting from the phenomenon, the terms actively and passively infected are introduced.

The vector efficiency of a vector group, composed of 10 apterae, is approximately 75 per cent, while that of a single aptera is approximately 25 per cent.

First instar nymphs, pupae, alate viviparae, and apterous viviparae will transmit the virus, and the virus can be retained through a moult.

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VARIABILITY OF THE CUCURBIT ROOT-ROT FUNGUS, FUSARIUM (HYPOMYCES) SOLANI F. CUCURBITAE[1]

N. PRASAD

Hypomyces solani Rke. et Berth. emend Snyd. et Hans. is the perfect stage of *Fusarium solani* (Mart.) Appel et Wr. emend Snyd. et Hans.; forms of which cause root rot of several important crops. The form parasitic on squash, *H. solani* f. *cucurbitae* Snyd. et Hans. has been shown by Hansen and Snyder (3) to be an extremely variable fungus. They have found numerous strains in culture and in nature which differ not only in pigmentation, rate and type of growth, but also in their sex and compatibility groups. Some of the strains are more pathogenic than others and are capable of attacking a number of cucurbits including melons and gourds. This study was undertaken to find out if any correlation existed between cultural variation and pathogenicity.

COMPARATIVE MORPHOLOGY OF STRAINS

Thirty-three strains of *Fusarium solani* f. *cucurbitae*, kindly supplied by Dr. H. N. Hansen, were maintained in a pure state on potato-dextrose agar by repeated single conidium transfer at frequent intervals following the method of Hansen and Smith (2).

The thirty-three strains can be grouped into seven distinct groups on the basis of pigmentation, production of sporodochia, amount of aerial mycelium and the type of growth on potato-dextrose agar. The slow, button type of growth is characteristic of strains 22, 25, 26, 28, 32, and 33. The thirty-three strains differ among themselves in pigmentation, from pure white to yellow, green and bluish-green, and in size and septation of the macro-conidia as shown in table 1.

The longest (61.7 μ) occurred in strain 30 and the shortest (20.2 μ) in strain 3. The shape of macro-conidia is one of the characters upon which Snyder and Hansen (4, 5, 6) have based the separation of species in the genus *Fusarium*. Macro-conidia of *F. solani* may have pointed or blunt ends and the degree of curvature varies. The macro-conidia of strain 3 show blunt ends and only slight curvature in contrast to the other strains. Anastomosis of the conidia *in situ* is characteristic of strains 7 and 19.

If one were to follow Wollenweber and Reinking's (7) classification of the Fusaria, based mostly on the size of macro-conidia, one could separate the thirty-three strains into five or six different species. This study shows that neither size of conidia nor a reasonable variation in shape can be relied upon as a morphological character in the determination of species in

Fusarium. Were every variation in conidial size or in other cultural charac-
ter used to distinguish species in this pathogen, the number of species
causing squash root rot would be in proportion to the number of variants
recovered. But since all 33 of the strains studied have been derived from
the perithecial stage (*Hypomyces*) of one fungus, it would seem more
logical to recognize as the normal variability of that fungus all variations
in cultural characters displayed by these 33 strains or by any additional
ones so derived. Some of the cultural variations are evident in figure 1.

TABLE 1.—*Range in microns of 50 macro-conidia taken at random from each of 33
different strains of Fusarium solani f. cucurbitae in cultures 3 weeks old*

Strain	Length	Width	No. of Septa
1	39.2–51.3	3.0–4.2	1–4
2	31.3–46.74	3.5–4.5	1–4
3	20.2–30.76	3.0–3.8	1–2
4	35.2–53.2	3.5–4.5	0–4
5	36.5–52.6	3.9–5.2	0–5
6	30.5–42.6	4.0–5.2	0–4
7	34.3–45.0	3.8–5.2	0–4
8	35.0–60.3	4.1–5.8	0–5
9	33.2–40.4	3.8–4.6	0–3
10	38.7–50.5	3.7–5.1	0–4
11	40.1–55.6	3.9–5.4	0–5
12	39.2–57.6	3.9–5.4	0–5
13	31.2–46.2	3.6–4.7	0–4
14	42.7–61.3	4.1–5.8	0–5
15	40.7–61.5	4.0–5.8	0–5
16	30.6–41.5	3.9–4.6	0–4
17	42.5–67.5	3.7–5.8	0–6
18	30.5–45.4	3.5–4.2	0–4
19	34.3–51.2	3.9–5.1	1–4
20	29.3–43.6	3.6–4.8	0–4
21	31.2–47.4	3.2–4.9	1–4
22	36.7–51.2	3.5–4.9	1–4
23	34.2–55.2	3.9–4.2	0–4
24	31.3–48.4	3.5–4.9	0–5
25	37.2–55.4	3.8–5.4	0–5
26	31.2–46.3	3.2–4.7	0–4
27	27.5–36.7	3.5–4.8	0–3
28	35.6–48.5	3.9–4.7	1–4
29	37.0–49.2	3.8–4.9	1–4
30	41.2–61.7	3.9–5.7	1–5
31	36.7–49.2	3.5–4.9	1–4
32	20.3–35.3	3.3–4.7	0–2
33	37.8–57.3	3.7–5.7	1–5

COMPARATIVE PATHOGENICITY OF STRAINS

Experiments to test the relative pathogenicity of these strains were
carried out in sterilized soil in the greenhouse. Two methods of inoculation
were followed. The first method consisted in dipping squash seed (Black
Zucchini, *Cucurbita pepo L.*) in a spore suspension of each of the thirty-
three strains of *Fusarium solani. f. cucurbitae* and planting in 6-inch pots
In the second method of inoculation the seeds of squash were sown in pots
and after the seedlings had emerged, spore suspensions from the different

strains were poured into the pots. To prevent contamination from one pot
to another by splashing of water, the pots were sub-irrigated.

With virulent strains, the symptoms of infection appeared in about
a week's time. The first symptom was the appearance of discoloration at
the foot which proceeded upward in the form of a streak in the cortical
region. The plants died within two days. With weakly parasitic strains,
the plants were not killed even ten days after the appearance of first
symptoms. On the basis of number of days required to kill the plants and
the extent of the lesions produced, the strains can be grouped as very
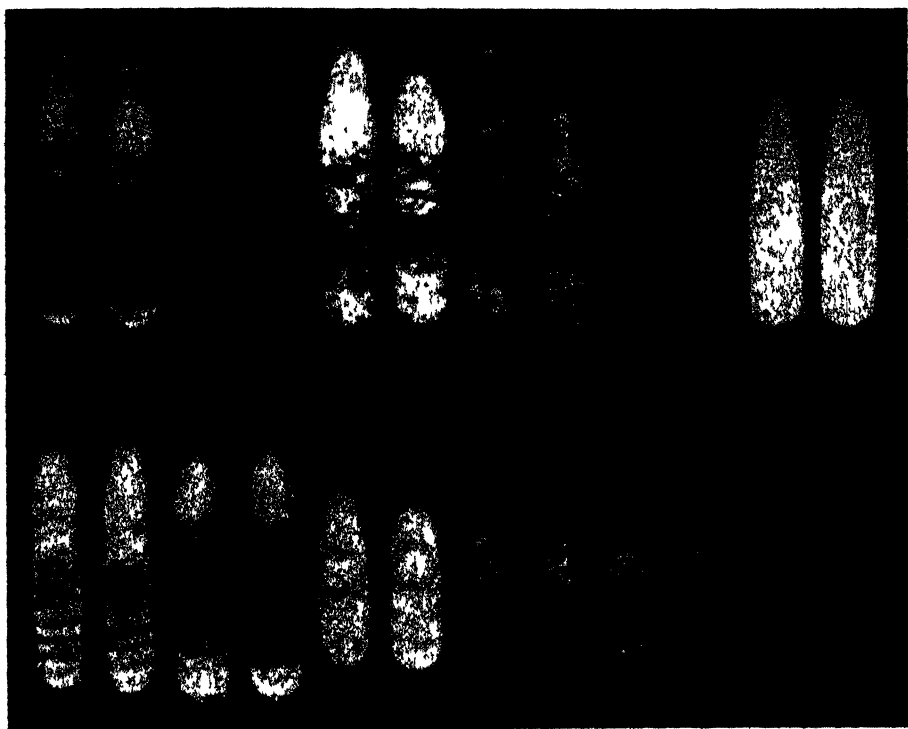
FIG. 1. Some of the strains of *Fusarium solani* f. *cucurbitae* showing variation in
the rate and type of growth and in pigmentation. All cultures were started from single
conidia and are of the same age and grown on the same medium.

virulent, virulent, fairly virulent, weak, very weak, and non-parasitic. The
strains 8, 12, and 19 are very virulent and killed the plants in 10 days.
Eighteen came under the category of virulent strains as they caused the
death of the plants in 12 to 15 days. There was only one strain, 33, which
could be called fairly virulent as it caused lesions in 10 days. Weak strains
22, 28, 29 caused lesions in 15 days. Strains 25 and 26 were classed as
very weak pathogens because only very slight lesions appeared after
20 days. Strain 32 was the only one which was non-parasitic under the
conditions of the experiments since it failed to produce lesions of any kind
even 30 days after inoculation.

RELATION OF PATHOGENICITY TO GROWTH RATE

To find out whether rate of growth had any relationship to pathogenicity, the five strains, 8, 12, 22, 25, and 33, were grown in U tubes designed by H. N. Hansen, each consisting of 2-cm. glass tubing, bent in a U fashion, with each arm 30 cm. in length and the ends bent upwards. These tubes were half-filled with potato-dextrose agar, sterilized, and cooled in a horizontal position (Fig. 2). They were inoculated at each end with a single spore, a different strain being used for each tube. After a few days the margin of growth was marked on each tube and measurements of subsequent
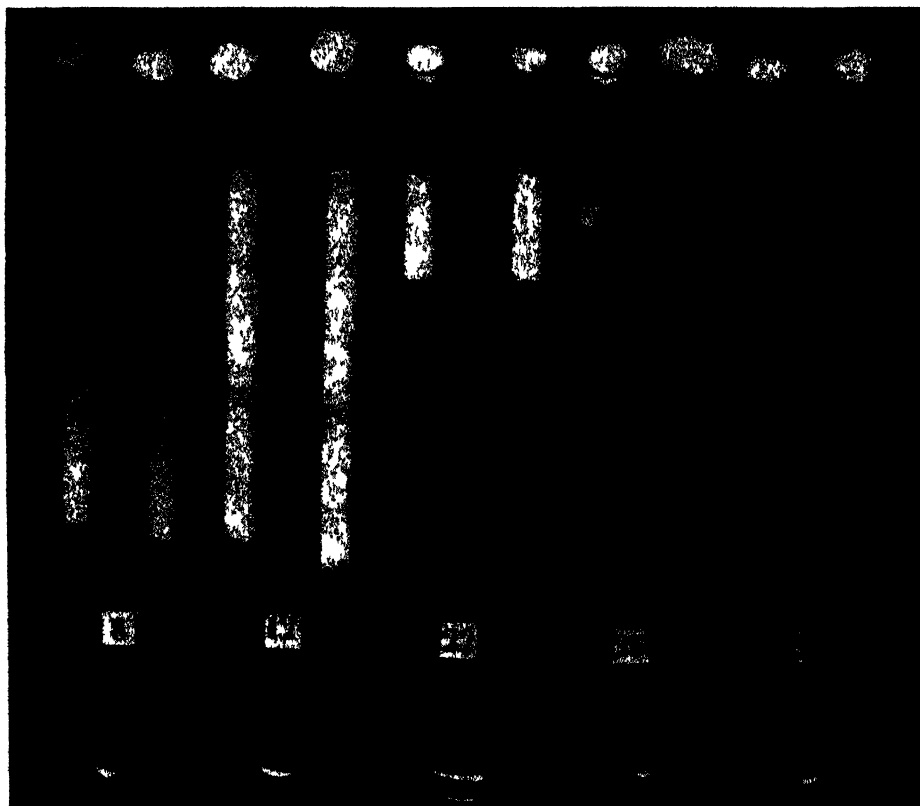
FIG. 2. Comparative rates of growth of strains 8, 12, 22, 25, and 33 when cultured simultaneously in U tubes under laboratory conditions. Strains 8 and 12 are the most rapid in growth and are the most virulent.

growth were taken from this line every day (Table 2). Strains 8 and 12 grow most rapidly and almost at the same rate and are very virulent. Strains 33 and 25 have the slowest rate of growth while strain 22 is intermediate and these 3 strains are less virulent than 8 and 12.

An infection experiment was conducted to find out if there was any closer correlation between rate of growth and pathogenicity. Seedlings were grown in pots and when they were about 3 inches tall, a spore suspension of the pathogen was poured into each pot. Spores from one culture tube

TABLE 2.—*Comparative rates of radial growth in millimeters of certain strains of Fusarium solani f. cucurbitae at room temperature in U-tubes*

Strain	Total growth in 2 weeks, mm.	Average rate of growth per day, mm.
8	86.8	6.2
12	88.0	6.3
22	23.8	1.7
25	17.0	0.5
33	10.0	0.4

where the fungus had grown for 3 weeks, were used for infecting one pot. The opaqueness of the spore suspensions was comparable in each case.

The results obtained (Table 3) show that the rapidly growing strains (8 and 12) are more pathogenic than 22, 25, and 33 which grow slowly. But strain 33 which grows at a slower rate than either 22 or 25 is more pathogenic than 22 or 25.

The comparison of the pathogenicity of different strains in this manner is open to criticism since it was not certain that exactly the same amount of inoculum was used in each case. A better method of comparison would be to bring about infection with a single spore of each strain and to measure the size of the lesions produced by each in a given period of time. This method would give a measure of the virulence of a strain in terms of time.

Single spore inoculation.—Single germinated spores were picked from water agar plates as used in the preparation of single spore cultures (5). The pieces of agar, measuring 1 to 2 mm. on a side, bearing single germinated spores were applied to the stems of potted squash seedlings. It was found that the pieces of agar dried up very quickly and that in the greenhouse infection could not be established by this method without some modification. It was necessary to keep the plants in a moist chamber long enough for the fungus to establish itself. It was found that with the fast growing strains, symptoms appeared by the eighth day after inoculation. However no symptoms appeared during this period where the slow-growing strains were tried.

In another test, seedlings were grown from sterilized seed in Petri dishes on moist filter paper sterilized by boiling. Six days after germination, a

TABLE 3.—*Rate of killing of squash plants when pots of seedlings were inoculated separately with strains 8, 12, 22, 25, and 33 of Fusarium solani f. cucurbitae, using comparable amounts of inoculum*

Strain of fungus	Total number of plants killed	Time required to kill the plants	General remarks
8	5	9 days	Plants completely rotted
12	5	9 days	Plants completely rotted
22	none	.	Lesions produced at the foot
25	none	.	Very slight lesions
33	1	15 days	Lesions well marked

small piece of agar containing a single germinated spore was placed on each seedling.   When the seedlings became too big for the Petri dishes, they were transferred to small Ehrlenmeyer flasks containing Hoagland's solution which was changed after every 4 days to prevent the growth of algae in the flasks.   Five squash seedlings were inoculated with single germinated spores from each of four strains 8, 12, 22, and 33.   The extent of the lesions produced by each strain was measured 6 days after inoculation (Table 4). Strains 8 and 12 produced the largest lesions, 15 × 5 mm. in size.   The lesions caused by strain 33 were much greater than those caused by strain 22 although the latter had a higher rate of growth than the former.   The lesions produced by these two strains measured 5 × 2 and 2 × 1 mm., respectively.   Seventeen days after inoculation the plants inoculated with strain 33 died, while strain 22 did not kill the plants.

From the above it appears that there is no consistent correlation between the rate of growth of the fungus and its pathogenicity.   Strains 8 and 12

TABLE 4.—*Comparison of pathogenicity of strains 8, 12, 22, and 33 of Fusarium solani f. cucurbitae obtained by inoculating squash plants with a single spore from each strain*

Strain	Rate of mycelial growth (in mm.) per day	Size of the lesion (in mm.) six days after inoculation	Time (in days) required to kill the plant
8	6.2	15 × 5	10
12	6.3	14 × 5	10
22	1.7	2 × 1	No death
33	0.4	5 × 2	17

are the fastest growing strains and the most virulent but strain 22, which grows more rapidly than 33, is not so pathogenic as 33.

NUTRITIONAL REQUIREMENTS OF CERTAIN STRAINS

Beadle and Tatum (1) found that single spore strains of *Neurospora crassa* Shear et Dodge differed in nutritional requirements.   Their work suggested that by providing proper vitamins to the different strains of *Fusarium solani* f. *cucurbitae,* it might be possible to make the slow-growing strains grow faster and show greater pathogenicity.

With that object in view, certain strains of *Fusarium solani* f. *cucurbitae* were grown on Beadle's (1) minimal medium and on potato-dextrose agar (P.D.A.) to which the following substances were added separately : thiamin, niacin, folic acid, pyridoxine, pantothenic acid, and biotin.   The amount of vitamins added per liter was as follows: thiamin 100 mg, niacin 200 mg, pyridoxine 50 mg, folic acid 15 mg, pantothenic acid 200 mg, and biotin 6×10^{-6} gm.   These are the concentrations used by Beadle.   Equal quantities of each of the above media were poured into Petri dishes and each of these was inoculated with a single spore of strains 8, 22, 25, and 33.   A line was marked with a wax pencil across each Petri dish and it was on

this line that the measurement of the diameter of each colony was taken daily for a period of two weeks (Table 5).

The results show that *Fusarium solani* f. *cucurbitae* does not grow well on synthetic media even in the presence of various vitamins. Growth of all the strains was much better on P.D.A. than on Beadle's medium.

Strain 8 normally grows well on P.D.A. and produces aerial mycelium and green pigmentation. It failed to produce any aerial mycelium or pigmentation when grown on Beadle's medium plus the vitamins. Strains 22 and 25 did not grow well on any one of the media, but 22 grew much better than 25. Strain 33 grew poorly on all media with the exception of P.D.A. to which 5×10^{-6} gm. per liter of biotin had been added.

TABLE 5.—*Growth of four strains of Fusarium solani f. cucurbitae on media containing different growth substances*

Medium	Average rate of growth of strains (in mm. per day)				Total growth (in mm.) of strain after 2 weeks			
	8	22	25	33	8	22	25	33
Beadle's minimal medium	2.0	0.5	0.2	0.2	28.0	7.0	2.8	2.8
Beadle's minimal medium + Thiamin	2.0	0.4	0.2	0.2	28.0	5.5	2.8	2.8
Beadle's minimal medium + Niacin	2.2	0.6	0.2	0.2	30.0	8.4	2.8	2.8
Beadle's medium + Pantothenic acid	2.1	0.5	0.15	0.13	29.0	7.0	2.0	1.8
Beadle's medium + Biotin	2.3	0.5	0.2	0.3	32.0	7.0	2.8	4.7
Beadle's medium + Pyridoxine	2.0	0.4	0.2	0.2	28.0	5.5	2.8	2.8
Beadle's medium + Folic acid	2.2	0.5	0.25	0.2	30.0	7.0	3.5	2.8
Potato-dextrose agar	6.3	1.7	0.5	0.4	88.0	24.0	7.0	8.0
Potato-dextrose agar + Thiamin	6.2	1.7	0.6	0.5	87.0	24.0	8.5	7.0
Potato-dextrose agar + Niacin	6.2	1.8	0.55	0.6	87.0	25.0	7.7	8.5
Potato-dextrose agar + Pantothenic acid	6.4	1.8	0.5	0.5	89.6	25.0	7.0	7.0
Potato-dextrose agar + Biotin	6.3	1.7	0.6	2.3	88.0	24.0	8.5	32.2
Potato-dextrose agar + Pyridoxine	6.2	1.6	0.5	0.4	87.0	22.0	7.0	5.5
Potato-dextrose agar + Folic acid	6.2	1.7	0.4	0.5	87.0	24.0	5.5	7.0

Normally, it grows very slowly in the form of a button on P.D.A., producing a bluish pigmentation and as it grows the medium becomes colored deep brown. But, when it was grown on P.D.A. to which biotin was added, instead of the characteristic button type of growth, it produced an increased amount of mycelium and resembled strain 8. The medium retained its semi-transparent nature. This experiment suggested that strain 33 required biotin for its normal growth and that the amount of biotin ordinarily present in P.D.A. was not sufficient for it to grow normally.

EFFECT OF BIOTIN ON PATHOGENICITY

The previous experiment suggested that biotin might increase the pathogenicity of strain 33 since an additional supply of biotin stimulated its growth in vitro. Squash seedlings grown in Petri dishes were inoculated with single germinated spores from strains 8 and 33, and after inoculation

were transferred to Ehrlenmeyer flasks containing Hoagland's solution. In one series of flasks, biotin was added at the rate of 10×10^{-6} gm. per liter of Hoagland's solution. It was observed that strain 33 was more pathogenic in the medium which contained the additional supply of biotin. The extent of lesions produced by strain 33 in the presence of the additional biotin, was much greater than in the absence of biotin. Although the addition of biotin increased the pathogenicity of strain 33, it was still less pathogenic than strain 8. This difference might be due to the inability of strain 33 to produce something else which plays as important a role as biotin.

DISCUSSION

The study of variation in size of macro-conidia within a single form of one species based on direct descendency, shows clearly that size of conidia is not a reliable character for the identification of species of *Fusarium.* If one were to base species on the measurement of conidia, it would be necessary to place the thirty-three strains of a single species into at least five or six different species. Wollenweber and Reinking (7) in their classification of Fusaria, have stressed the importance of size of macro-conidia and production of pigment among other characters. Evidence presented here indicates that these characters are not dependable for the identification of Fusaria. These studies support Snyder and Hansen's (4, 5, 6) system of classification of Fusaria wherein they do not base their species on the measurement of macro-conidia but regard the differences in measurements as normal variation between clones of the same species. One is convinced of the usefulness of their system of classification when one examines the variation in size and in production of sporodochia, sclerotia, and pigment within the thirty-three strains of *F. solani* f. cucurbitae.

Since different strains of *Fusarium solani* f. *cucurbitae* have different rates of growth, one is tempted to correlate pathogenicity with rate of growth. Strains which have a higher rate of growth should be more pathogenic than the slow growing ones. Strains 8 and 12 grow fastest and are also most pathogenic But this has not been found to be true when other strains are compared for pathogenicity. Strain 33 which grows at a slower rate than strains 22, 25, 26, or 28, has been found to be more pathogenic. This behavior was more apparent with single spore inoculations. This method of inoculation has proved to be a very reliable and practical method of comparing the different strains of *F. solani* f. *cucurbitae* for pathogenicity.

SUMMARY

Thirty-three strains of *Fusarium solani* f. *cucurbitae* were found to differ from each other in culture type, rate of growth, pigmentation and size of macro-conidia.

Inoculation of the host plant (squash seedling) with a single germinated spore provided a satisfactory method for the comparison of different strains

with respect to pathogenicity. The strains were found to vary greatly in pathogenicity but consistent correlation was not found between rate of growth and pathogenicity. Strains 8 and 12 are the fastest growing strains and also the most highly pathogenic. Strain 33 which has a slower rate of growth than strains 22 and 25, was found to be more pathogenic than either strain 22 or 25.

Rate of growth of strain 33 was increased with additional amounts of biotin in the medium. It also showed greater pathogenicity in the presence of an additional supply of biotin.

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THE OCCURRENCE OF PUCCINIA CORONATA ON RHAMNUS FRANGULA IN CANADA[1]

B. PETURSON[2]

Crown rust (*Puccinia coronata* Cda.) has been studied extensively in both Europe and America. These studies have shown that this rust is composed of a large number of morphologically similar strains or varieties that have their aecial stages on several species of the Rhamnaceae and Elaeagnaceae and their uredial and telial stages on various grasses. In Europe, the aecial stages of crown rust varieties occur most commonly either on *Rhamnus cathartica* L. or *R. Frangula* L. A number of the European crown rust varieties that occur on *R. cathartica* apparently also occur in America. However, none of those that occur on *R. Frangula* has hitherto been reported to occur on that host in North America, although the aecial host grows commonly in many localities in the United States and Canada.

Since 1939, aecial infections of a variety of crown rust have been collected every spring on *Rhamnus Frangula* at Fredericton, N. B. Telial infections of this rust have also been collected in three different years on *Agrostis* spp. growing in the vicinity of the infected *R. Frangula* bushes. This rust has been cultured on its aecial host, *R. Frangula*, and on several species of its gramineous host under greenhouse conditions at the Dominion Laboratory of Plant Pathology, Winnipeg. A description of this crown rust variety is given in the present paper.

LITERATURE REVIEW

The heteroecious nature of crown rust was demonstrated in 1865 by De Bary (5), who infected the leaves of two European species of *Rhamnus*, *R. Frangula* and *R. cathartica*, with teliospores of crown rust collected on grasses.

In 1874, Nielsen (18) obtained heavy uredial infection on *Lolium perenne* L. with aeciospores from *Rhamnus cathartica*, and with the urediospores thus obtained on this grass he infected cultivated oats. He could not, however, cause infection on *L. perenne* with the aeciospores that occurred on *R. Frangula* and, therefore, came to the conclusion that two rusts were included under the name *Puccinia coronata*. To the rust that had its aecial stage on *R. cathartica* and its uredial and telial stages on *L. perenne* and cultivated oats he gave the name *P. Lolii* Nielsen.

Experiments carried out by Plowright (19) in 1889 further demonstrated the difference between the varieties of crown rust that had their

aecial stages respectively on *Rhamnus cathartica* and *R. Frangula*. He considered that two species of crown rust were being confounded under the name *Puccinia coronata*.

In 1894, Klebahn (10) found that the crown rusts occurring on *Avena sativa* L., *Arrenatherum elatius* (L.) Mert. and Koch, and *Lolium perenne* could infect *Rhamnus cathartica* but not *R. Frangula*. Klebahn, like Nielsen and Plowright, came to the conclusion that there were two crown rust species present and, to the rust on *R. Frangula*, he gave the name *Puccinia coronata* (Corda) Kleb. The rust on *R. cathartica* he named *P. coronifera* Kleb. He was apparently unwilling to accept the name *P. Lolii* Niels. that Nielsen had previously given to this rust.

Eriksson (8) and Klebahn (11, 12) found that the crown rusts were highly specialized in respect to their gramineous hosts and listed several varieties (*formae speciales*) of each rust. Their final arrangement of the varieties listed was as follows:

Puccinia coronata with aecia on *Rhamnus Frangula*

1. var. *Agropyri* Erikss.—on *Agropyron repens* (L.) Beauv.
2. var. *Agrostis* Erikss.—on *Agrostis stolonifera* L. and *A. vulgaris* With.
3. var. *Calamagrostis* Erikss.—on *Calamagrostis lanceolata* Roth.
4. var. *Holci* Kleb.—on *Holcus mollis* L. and *H. lanatus* L.
5. var. *Phalaris* Kleb.—on *Phalaris arundinacea* Roth.

Puccinia coronifera with aecia on *R. cathartica*

1. var. *Avenae* Erikss.—on *Avena sativa* L.
2. var. *Alopecuri* Erikss.—on *Alopecurus pratensis* L.
3. var. *Agropyri* Erikss.—on *Agropyron repens* (L.) Beauv. only.
4. var. *Epigaei* Erikss.—on *Calamagrostis epigeios* (L.) Roth.
5. var. *Festucae* Erikss.—on *Festuca elatior* L. and *F. gigantea* (L.) Vill.
6. var. *Glyceriae* Erikss.—on *Glyceria aquatica* Wahlb.
7. var. *Holci* Kleb.—on *Holcus lanatus* L. only.
8. var. *Lolii* Erikss.—on *Lolium perenne* L.

Muehlethaler (15, 16), in 1911, added to this latter group a ninth variety, *Bromi*, which occurs on several species of *Bromus*; and Brown (3), in 1947, isolated and described the variety *Arrhenatheri*, which was collected on *Arrhenatherum avenaceum* Beauv.

In America, Arthur and Holway (2) in 1898, and Carleton (4) in 1899 succeeded in producing aecial infections on the leaves of *Rhamnus lanceolata* Pursh., a common native species of the southern United States. They made their inoculations with teliospores from cultivated oats. Later, Arthur (1) obtained aecial infection on the leaves of *R. alnifolia* L'Her., a northern native *Rhamnus* species, with teliospores from *Calamagrostis canadensis* (Michx.) Beauv. Both Arthur and Carleton disagreed with Nielsen's and with Klebahn's nomenclature of crown rust. They doubted the existence, at least in America, of two crown rust species, and therefore referred to the crown rust occurring in America as *Puccinia coronata* Cda.

Melhus and Durell (13) found, in 1915, that teliospores on oats could cause heavy aecial infections on *Rhamnus cathartica* but none on *R. Frangula*.

Melhus et al. (14) tested several species of *Rhamnus* in 1919, 1920, and 1921, to crown rust occurring on oats, *Calamagrostis canadensis*, and *Festuca elatior*. In these tests, *R. Frangula* proved to be highly resistant to the rust on all these gramineous hosts.

Deitz (6, 7), working at Ames, Iowa, obtained in a few of his trials several pycnial infections, but never aecial infections, on *Rhamnus Frangula* when inoculations were made with teliospores from oats. He kept under observation for the 8-year period, 1916 to 1924, a number of bushes of *R. Frangula* growing on the campus at Ames, but at no time found pycnia or aecia on these bushes, although bushes of *R. cathartica* growing nearby became infected each year. In 1923 and 1924, he kept also under observation ten plantings of *R. Frangula* in the New England States. No aecia were found on these plantings, whereas aecial infections were abundant on *R. cathartica* in that area. In experiments carried out at Ames, Iowa, in 1929, Murphy (17) failed to obtain infection on *R. Frangula* with teliospores from oats. In these same tests he obtained normal spermogonia and aecia on *R. cathartica* with the teliospore material that failed to cause infection on *R. Frangula*.

Fraser and Ledingham (9), in 1933, collected and studied four distinct varieties of crown rust at Saskatoon, Saskatchewan, Canada. These were as follows:

Var. *Avenae*—with aecia on *Rhamnus cathartica*.
Var. *Calamagrostis*—with aecia on *R. alnifolia*.
Var. *Elaeagni*—with aecia on *Elaeagnus commutata* Bernh.
Var. *Bromi*—with aecia on *Lepargyraea canadensis* (L.). Greene.

The variety *Bromi* described by Fraser and Ledingham failed to infect *Rhamnus cathartica* and evidently was distinct from the variety *Bromi* described by Muehlethaler in Europe. Fraser kept under observation for several years specimens of *R. Frangula* growing at Saskatoon, but failed to find any rust infections on that host.

EXPERIMENTAL WORK

Heavy aecial infections were observed on *Rhamnus Frangula* at Fredericton, N. B., in 1939 by Mr. S. F. Clarkson and Mr. J. L. Howatt, of the Dominion Laboratory of Plant Pathology, Fredericton, N. B. So far as the author has been able to ascertain, this was the first recorded observation of the occurrence of aecial infection on *R. Frangula* in America and, to date aecial infection on this host has not been reported elsewhere in North America. This rust has been observed and collected on *R. Frangula* at Fredericton every year since 1939.

A search was made in the vicinity of the infected buckthorn bushes for the telial stage of the rust occurring on them and abundant telial infections

of a crown rust were found nearby on a grass identified as an *Agrostis* sp. Several collections of the teliospores occurring on this grass and of the aecia occurring on *Rhamnus Frangula* were sent to the Dominion Laboratory of Plant Pathology, Winnipeg, Manitoba for further study.

In the tests that were made, the aeciospores from the infections on *Rhamnus Frangula* failed to cause infection on cultivated oats but normally infected seedling plants of several species of *Agrostis*. Coronate teliospores were formed on the grass hosts in the greenhouse. These teliospores, when used for the inoculation of *R. Frangula*, caused normal pycnia and aecia to form. The teliospores found on *Agrostis* in the vicinity of the infected

TABLE 1.—*The reactions of oats and 19 grass species to the rust occurring on Rhamnus Frangula*

Host	Rust reaction
Agrostis hyemalis Walt.	Susceptible
A. tenuis Sibth. `(A. vulgaris* With.)	do
A. lacnantha Nees.	do
A. nebulosa Boiss. and Rent.	do
A. rupestris Champ.	do
A. stolonifera L.	Moderately susceptible
Calamagrostis canadensis (Michx.) Beauv.	Moderately resistant
C. arundinacea Roth.	do
Agrostis alba L.	Immune
A. spica venti L.	do
A. verticillata Vill.	do
A. canina L.	do
Agropyron repens (L.) Beauv.	do
Avena sativa L. (Victory)	do
Calamagrostis lanceolata Roth.	do
Dactylis glomerata L.	do
Holcus lanatus L.	do
Lolium multiflorum Lam.	do
L. perenne L.	do
Phalaris arundinacea L.	do

bushes of *R. Frangula* germinated readily and caused heavy aecial production on *R. Frangula*, but failed to infect *R. cathartica*.

Cultivated oats and a number of grasses were inoculated in the seedling stage with aeciospores from *Rhamnus Frangula*. Each species investigated was subjected to several different inoculation tests with aeciospores. Later, the oats and grasses were all inoculated with the urediospores that resulted on the susceptible grasses from the infections caused by the aeciospores. Table 1 gives the rust reactions of the grass species tested to the rust occurring on *R. Frangula*.

The behavior of this rust on the grass hosts tested shows it to be identical with a variety of crown rust that Eriksson (8) isolated in Europe in 1897 and named *Puccinia coronata* Corda forma *Agrostis* Erikss. The rust variety isolated by Eriksson, like the one under study, had its aecial stage on *R. Frangula* and its uredial and telial stages on *Agrostis vulgaris* (*A. tenuis*), *A. stolonifera,* and other grasses.

The aecia on *Rhamnus Frangula* occur on the petioles and on the under

side of the leaves, with the majority of the aecia confined to the leaves. The aecial pustules vary from round to oval, according to their location on the leaves. The ones that form between the leaf veins are usually small and circular, whereas those that form on the leaf veins are larger and oval, their oval shape being due to the tendency of the pustules to spread along the leaf veins. Some hypertrophy of the host tissues is associated with aecial formation, particularly when the aecia occur on the leaf petioles. Measurements were taken of aecial cups formed outdoors at Fredericton in 1947. The average external diameter of the 120 cups measured was 247 microns, and they ranged in size from 197 to 329 microns in external diameter.

Measurements were made of the aeciospores, urediospores, and teliospores of *Puccinia coronata* var. *Agrostis*. The aeciospores measured were taken from field collections obtained at Fredericton, N. B., in 1947, and the urediospores and teliospores measured were formed on *Agrostis tenuis* in the greenhouse at Winnipeg in 1947. In measuring the aeciospores and

TABLE 2.—*The minimum, maximum, and mean length and width of aeciospores, urediospores, and teliospores of Puccinia coronata var. Agrostis*

Spore form	Spore length in microns			Spore width in microns			No. of spores measured
	Minimum	Maximum	Average	Minimum	Maximum	Average	
Aeciospore	15.5	25.7	20.1	13.3	20.4	17.3	100
Urediospore	17.7	26.6	21.2	14.2	25.7	19.5	100
Teliospore	31.5	60.9	40.8	12.6	23.1	16.8	200

urediospores, the greater spore diameter was taken as the spore length, and the lesser spore diameter as the spore width. The teliospores were measured for length from the attachment of the pedicel to the exterior of the apex of the upper cells. Their width was measured across the apex of the upper cell. The spore measurements are in table 2.

The average spore sizes recorded in table 2 for aeciospores, urediospores, and teliospores are almost the same as the spore sizes recorded by Fraser and Ledingham (9) for the crown rust variety that they designated as *Puccinia coronata* var. *Bromi*, but are definitely smaller than the spore sizes recorded by these authors for *P. coronata* var. *Avenae* and *P. coronata* var. *Elaeagni*.

The average length and width of the teliospores of *Puccinia coronata* var. *Agrostis*, as given by Eriksson (8), agree very closely with those of the rust under study. However, the range in spore length (38–44 μ) recorded by Eriksson was much less than that shown in table 2.

The teliospores varied greatly in size and shape, as is shown in figure 1.

DISCUSSION

Although *Puccinia coronata* var. *Agrostis* has not been found on its aecial host anywhere in America, except at Fredericton, N. B., it probably

occurs in the uredial and telial stages on its grass hosts elsewhere in America. Mr. I. L. Conners, Division of Botany and Plant Pathology, Department of Agriculture, Ottawa, states that three collections of crown rust on *Agrostis stolonifera*—one from Kentville, N S., and two from near Chelsea, Que.— are now in the Mycological Herbarium at Ottawa Freeman Weiss (20) has recorded the occurrence of a crown rust on *A. alba* L , *A. canina* L., *A. diegoensis* Vasey, *A. exarata* Trin., *A. palustris* Huds , and *A. tenuis* in many localities in the United States The exact identity of the variety or varieties of crown rust occurring on these grass hosts cannot, however, be established until the appropriate aecial-host inoculation tests have been carried out.

FIG 1.   Teliospores of *Puccinia coronata* var. *Agrostis* (×350).

Since the aecial host of this rust, *Rhamnus Frangula*, as well as several of its uredial and telial hosts, *Agrostis* spp , are native European species, it is probable that the rust has been introduced into America from Europe.

SUMMARY

The aecial stage of a European crown rust, *Puccinia coronata* var. *Agrostis*, has been collected on *R. Frangula* at Fredericton, N B. each year since 1939.

The telial and uredial stages of this rust were collected on *Agrostis* spp. in 1945, 1946, and 1947, growing in the vicinity of infected bushes of *Rhamnus Frangula*.

This rust can infect some, but not all, species of *Agrostis*.

Aeciospores, urediospores, and teliospores of *Puccinia coronata* var. *Agrostis* are somewhat smaller than the corresponding spores of *P. coronata*

var. *Avenae,* but are almost the same size as those of some other varieties of crown rust.

To date, aecial infections have not been reported on *Rhamnus Frangula* anywhere in North America, except at Fredericton, N. B.

ACKNOWLEDGMENT

The writer is greatly indebted to Messrs. S. F. Clarkson, Director, Plant Protection Service, Department of Agriculture, Fredericton, N.B., and J. L. Howatt, Associate Plant Pathologist, Dominion Laboratory of Plant Pathology, Fredericton, N.B., who drew his attention to the presence in Canada of the aecial stage of this rust and supplied field collections of it each summer during the course of the study.

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SYNCHYTRIUM FOUND ON THE ROOTS OF STRAWBERRY[1]

S. M. ZELLER AND LEO CAMPBELL

In March, 1946, small galls were discovered on the roots of Marshall strawberry plants grown near Lynden, Whatcom County, Washington. Inspectors first thought these to be caused by root-knot nematode but an expert nematologist found no parasitic nemas present. When some of the galls were crushed, however, sori of a *Synchytrium* were discovered.

The root galls are mostly less than 1 mm. in diameter and 2–4 mm. long. They may be lateral growths on rootlets or when within the rootlet itself they usually distort the tissues, forming a hook-shaped gall.

Normal growth of strawberry plants does not seem to be affected by the infection.

The fungus sori for the most part are on the inner curve of the hooked galls or on the surface of the gall next to the rootlet from which a lateral gall is produced.

The sori are rather bright yellow and when crushed may be seen to possess several sporangia. The sporangia are rather thin-walled, about $50–60 \times 60–80 \mu$ in diameter. At the time observed most of the sporangia were full of swarm spores which were very motile when liberated. The swarm spores are spherical, uniciliate, uninucleate, and about $1.5–2 \mu$ in diameter.

No *Synchytrium* has been reported on the roots of strawberry. Sey-mour[2] lists *Synchytrium globosum* on *Fragaria* (under *Pycnochytrium*), and *S. aureum* on *Fragaria virginiana*. Dr. Freeman Weiss of the U. S. Plant Disease Survey writes that all of the material of these two species in the Mycological Collections at Beltsville "consists of leaves or stems and these are the host parts that are characteristically infected by *Synchytrium* spp. In fact, I do not know of any record of Synchytrium galls on roots. . . . So, your Synchytrium gall on strawberry roots appears to be a novelty indeed."

Whether this organism was introduced into this particular strawberry field through some previous crop is not known. The land was first cleared in 1939 of Douglas fir, alder, etc., and planted to strawberries that year and the following three years. In 1943 and 1944 there were green manure crops of rye and vetch, and red clover, and then strawberries again in 1945.

[2] Seymour, A. B. Host Index of the Fungi of North America. Cambridge, Mass. 1929. (See p. 387.)

We believe this *Synchytrium* differs from other described species and therefore is presented here as **Synchytrium fragariae** sp. nov.

Soris multisporangiatibus, contentu flavo; zoosporangiis tenuo-muriatis, ellipsoideis vel subglobosis, vel subcuboideis, 50–60 × 60-80 μ crassis, e zoosporam maturitas farctis; zoosporis sphaeroideis, 1.5–2 μ crassis, 1-ciliatis, uninucleatis.

In gallis noduloideis in radicis parvis Fragariae, prope Lynden, Washington, Amer. bor.

Sorus multisporangiate, light yellowish; zoosporangia thin-walled, ellipsoid to subglobose, sometimes with flattened sides just after liberation, 50–60 × 60–80 μ in diameter, filled with swarm spores at maturity; swarm spores spherical, 1.5–2 μ in diameter, uniciliate, uninucleate.

Type locality: Near Lynden, Washington.
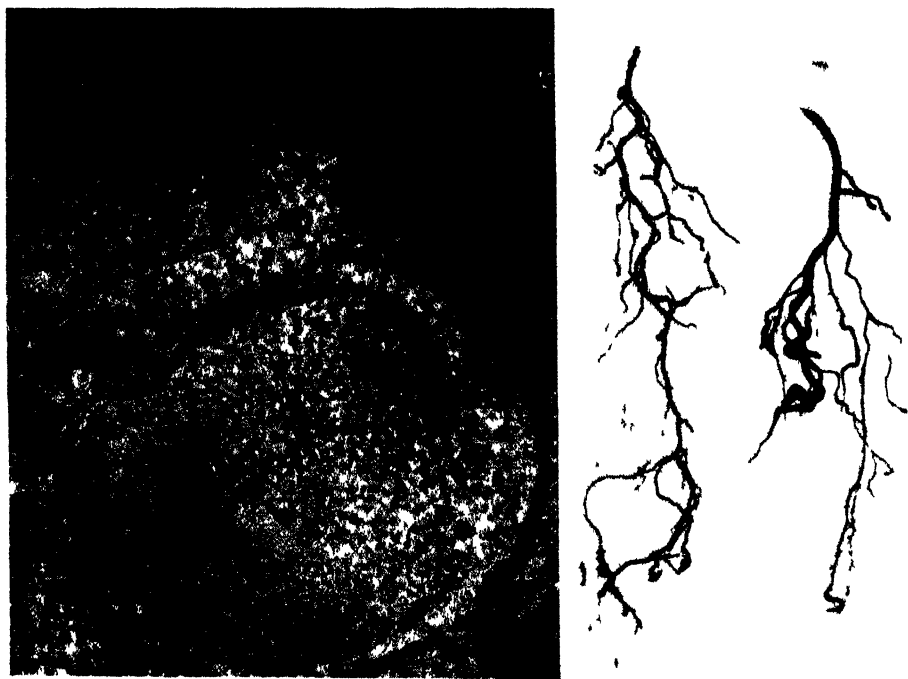
FIG. 1. Synchytrium on strawberry roots. Right, Strawberry roots with galls caused by *Synchytrium fragariae*, × 1; left, zoosporangia showing contents of zoospores (swarm spores), × 1000.

Habitat: In tiny nodule-like galls on roots of cultivated strawberry (*Fragaria*).

Distribution: Known from the type locality only, but by correspondence Dr. Gerald Thorne, Nematologist, U. S. Department of Agriculture, stationed at Salt Lake City, Utah, says the galls are "apparently the same as I found on some material from northern California several years ago," and the cause of which was not known. In that case the causal organism was not seen.

SUMMARY

A new species of *Synchytrium* found to cause tiny, nodule-like galls on the roots of cultivated strawberry (*Fragaria*), is described as *Synchytrium fragariae*. The type locality is near Lynden, Whatcom County, Washington.

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APPLICATION OF THE BACTERIOPHAGE-LYSIS TECHNIQUE FOR THE IDENTIFICATION OF PLANT PATHOGENIC BACTERIA

H. H. THORNBERRY,[1] A. C. BRAUN,[2] AND R. P. ELROD[3]

The specificity of bacteriophage lysis and the simplicity of the procedure for detecting lysis affords a practical means by which to identify bacterial plant pathogens and to diagnose bacterial plant diseases. The potentiality of the technique with plant pathogens has been mentioned by Coons and Kotila (2) and by Thomas (8, 11, 12) who also successfully identified species and strains of bacterial plant pathogens by this method (2, 6, 7, 9, 10). The two bacterial plant pathogens *Xanthomonas pruni* (E. F. Smith) Dowson and *Xanthomonas lactucae-scariolae* (Thornberry and Anderson) Burkholder which have been distinguishable only by pathogenicity (13) now may be distinguished by this technique (14, 15) when *X. pruni* bacteriophage (1) is used. With a sizeable volume of stored sterile *X. pruni* bacteriophage (15) and a large available collection of Xanthomonas species and cultures[4] used in extensive studies on the serological characterization of these organisms (3, 4, 5), a cooperative test on the lytic power of *X. pruni* bacteriophage on these bacteria was undertaken. The phage and directions for the technique were supplied by the senior author. The procedures were carried out and the results supplied by the junior authors.

The procedure used for the technique is as follows:

1. Prepare three tubes of young cultures showing turbidity upon agitation (24 to 48 hours' growth in 5-ml. broth tubes at pH 7.2 to 7.4 containing 0.2 per cent glucose—low glucose prevents the production of sufficient acid to interfere with lysis).

2. Inoculate two young culture tubes each with 0.5 ml. of phage inoculum and agitate for mixing, leaving one noninoculated young culture tube for control.

3. Incubate for 12 to 24 hours at 22°–24° C.

4. Observe relative turbidity of the agitated tubes at intervals. Lysis is demonstrated by relative decrease in turbidity when agitated.

The results show that *Xanthomonas pruni* bacteriophage causes a lysis in the six isolates of *X. pruni* (pruni 1, pruni 2, XP9, XP11, XP13, XP27)

but no definite reaction in the other species and their isolates.   One culture
of *X. manihotis* (XM12) and one of *X. phaseoli* (XP14) showed a ques-
tionable reaction which may have been due to partial inhibition of growth
by the filterable non-phage products of lysed *X. pruni* cells in the impure
phage inoculum.   However, no evidence of lysis was obtained with other
cultures of these two isolates.   Lysis was nil for all other tested isolates of
X. manihotis (XM6S, XM11) and other species and their isolates (*X. bar-
bareae* XB1 and XB2; *X. begoniae* XB3, XB8M, and XB8S; *X. beticola*
XB4; *X. campestris* XC2, XC3, XC13, and XC15; *X. campestris* var. *ar-
moraciae* Armoraciae 1; *X. corylina* XC5, XC8M, and XC12R; *X. cucur-
bitae* XC6; *X. geranii* XG4 and XG6; *X. holcicola* XH3; *X. juglandis*
XJ1, XJ3, XJ4, XJ5, and XJ5S; *X. lespedezae* XL1, XL2M, and XL2S;
X. malvacearum XM2M and XM13; *X. papavericola* XP5, XP17; *X.
pelargonii* XP15 and XP7; *X. phaseoli* var. *fuscans* XP26; *X. phaseoli*
var. *sojense* XP21; *X. rubrilineans* XR1 and XR2; *X. taraxaci* XT11;
X. translucens and its forms XT1, XT4, XT7, XT13, XT15, XT16, and
3061; *X. vesicatoria* XV4, XV8, and XV21; *X. vesicatoria* var. *raphani*
XV16; *X. vignicola* XV18, XV18S, and XV20; and *X. vitians* XV2).   The
results presented do not exclude the possibility that the isolates of resistant
species of the xanthomonads tested were resistant as a result of earlier in-
fection by this or by some other closely related strain of bacteriophage.
However, this appears unlikely since all *X. pruni* isolates tested responded
with lysis.

The lysis of bacteria by this strain of *Xanthomonas pruni* bacteriophage
is specific.   If there exist other strains of *X. pruni* bacteriophage with non-
specific lytic capacity or other bacteriophages and strains that lyse *X. pruni*
as well as other species of bacteria, such bacteriophages and strains would
not be useful for the purpose of simple direct identification of bacteria but
might be utilized for identifications through comparative lysis.   *Xantho-
monas pruni* bacteriophage is apparently more specific than are the cross-
agglutination tests.   There was no evidence of lysis with *X. corylina*, *X.
phaseoli* v. *sojense*, and *X. lespedezae*, all of which made a compact sero-
logical group with *X. pruni* (3).

The phage-lysis method has promise as a general identification pro-
cedure if highly specific phages for additional or all pathogens can be
found and preserved.   The lysis technique, once the phage is prepared, is
much simpler and faster than the usual staining, physiological, and sero-
logical procedures employed for identification.   Whether the specificity of
lysis parallels pathogenicity, genetic origin, any biochemical or serological
property of any organism is unanswered to date but appears to be im-
portant.   The success with the technique herein confirms previous claims.

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CHEMICAL SOIL TREATMENT FOR BLACK ROOT ROT OF TOBACCO IN THE GREENHOUSE

ROBERT A. STEINBERG AND E. E. CLAYTON[1]

Tobacco growers almost universally practice some method of soil sterilization in preparing a plant bed area. The primary objective is the destruction of weed seeds, but the elimination of soil borne diseases also is important. Chemical methods of soil sterilization have come into increasing use and one of the newest chemicals is urea.[2] At rates of ½ to 1¼ lb. per square yard of bed surface, various diseases, black root rot (*Thielaviopsis basicola* (Berk.) Ferraris),[3] root knot (*Heterodera marioni* (Cornu) Goodey),[4] and bacterial wilt (*Bacterium solanacearum* E. F. S.),[5] have been controlled. Results have been most satisfactory on the light sandy soils of the Southeast, and less so on the heavier soils where leaching is less rapid. It seems desirable to continue to search for more effective chemicals, particularly for use with heavy soils. The observations here reported included tests on the effectiveness of about 25 chemicals for use in soil sterilization against Thielaviopsis root rot of tobacco. The work was conducted during the winters of 1943 to 1947, inclusive.

One-gallon crocks with four seedlings of Maryland Broadleaf tobacco per crock were used in triplicate for each test. Each crock, except those of the healthy control, was filled with a half and half mixture of infested and sterilized soil into which the indicated chemical had been thoroughly incorporated. The treated soil was then held for a month or more in a moist condition before the tobacco seedlings were transplanted. The seedlings were lifted and their roots examined after approximately six weeks. Usually the effectiveness of the chemicals was further tested by planting and examining a second crop of seedlings. Between crops the soil was leached by several thorough waterings. Temperatures in the greenhouse were 50°–60° F. during the night; and 65°–75° F. during the day. Initial toxicity of the chemical to the plants would be a major factor with the first crop. The second crop would indicate the ease with which leaching might eliminate toxic chemical residues. Compounds were tried initially at the rate of 12 gm. per crock (1 lb. per sq. yd. of soil), and decreased in

quantity in subsequent trials until decisive results were obtained. Tests with the more promising compounds were repeated.

Of the compounds investigated urea, sodium azide, hexamethylene-tetramine, and sodium nitrite appeared most promising (Table 1). The compounds found to be unsatisfactory for partial soil sterilization against Thielaviopsis root-rot were calcium cyanamide, sodium formaldehyde sulfoxylate, hydrazine sulfate, hydroxylamine sulfate, 2,4-dinitro-diphenylamine, dicyandiamide, dipicrylamine, 2,4 diaminodiphenylamine, 4,4-diaminodiphenylamine, p,p-diamino diphenylmethane, s-diphenylthiourea, di-(B-hydroxyethyl) aniline, sodium salicylanilide, thymol, methyl violet, nile blue, malachite green, chloramin T, potassium ferrocyanide, and po-

TABLE 1.—*Control of black root rot (Thielaviopsis basicola) of tobacco by chemical treatment*

Chemical	Grams per Crock[a]	First Crop Plants		Second Crop Plants	
		Diseased	Healthy	Diseased	Healthy
		Number	Number	Number	Number
Check (Inoculated, not treated)	....	12	0	12	0
Check, Healthy	.. .	0	12	0	12
Urea	12	0	9[b]	3	7
Do	6	2	8[c]	1	11
Hexamethylenetetramine	12	0	0	0	11
Do	9	3	9[c]	4	8
Do	6	0	4	4	4
Sodium azide	1.0	0	0	0	12[b]
Do	0.8	0	12[b]	.. ...	.. ...
Do	0.6	0	12[b]	..	.. ...
Do	0.5	0	10[b]	4	8
Sodium nitrite	12	0	6[c]	0	5
Do	6	4	8[c]	.. ...	... ..

[a] Number of pounds to be applied to a square yard of bed surface is equivalent to 1/12 number of grams per crock.
[b] Growth of tobacco greatly retarded as a result of chemical toxicity.
[c] Growth of tobacco slightly retarded as a result of chemical toxicity.

tassium ferricyanide. The last 2 compounds were much more injurious to tobacco than to the fungus; stunting of the seedlings and mottling of the leaves occurred with as little as 1.5 gm. per crock, and the injury persisted after leaching.

Urea at 12 gm. per crock (1 lb. per sq. yd.) and frequently at 6 gm., gave satisfactory protection with first crop seedlings. The plants were usually slightly stunted as compared to the healthy controls, and some were killed. Light infection of some of the roots of the second planting usually could be found.

Sodium nitrite appeared to be more effective than urea on replanting, but was rather toxic to the seedlings unless leached from the soil.

Hexamethylenetetramine gave some control at 9 gm. per crock in both tests. It was more toxic than urea to tobacco and adequate leaching is desirable. Stunting or killing of seedlings was evident with first crop

seedlings. This compound is used in human medicine and is relatively non-toxic to animals.

Sodium azide proved most effective of all the chemicals tested Quantities of 0.5 to 1.0 gm. per crock gave good root-rot control with both crops of seedlings. Without leaching, the first crop of plants was killed; with leaching, 1.0 gm. per crock gave excellent disease control, but growth of tobacco was retarded. This compound is very poisonous to animals and it would be inadvisable to recommend its use until the hazards have been eliminated. It forms explosive compounds with metals such as copper.

Urea is the least expensive of the effective compounds, and it also has some value as a source of nitrogen. An attempt was made, therefore, to improve the effectiveness of urea through admixture of other compounds, particularly sodium azide, hexamethylenetetramine, and dichloramin T. No synergistic effects could be detected.

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SCLEROTINIA TRIFOLIORUM, A PATHOGEN OF LADINO CLOVER[1]

K. W. Kreitlow[2]

INTRODUCTION

Winter-survival is one of the major problems confronting growers of the Ladino variety of clover (*Trifolium repens* L.). Damage to old plants is usually most evident in the spring and is frequently attributed to winter-injury. Observations indicate the probable involvement of a number of factors including a destructive disease caused by *Sclerotinia trifoliorum* Erikss.

In northern Europe, *Sclerotinia trifoliorum* has long been recognized as an important pathogen of forage legumes. *Trifolium repens*, however, is usually considered resistant. Pape (5), failed to observe *Sclerotinia* attacking white clover, but he cites several authors who observed minor attacks on this host. Frandsen (2), inoculated *T. repens* with *Sclerotinia trifoliorum* and found it was not infected so readily as other species. In Kentucky, Gilbert and Bennett (3), observed *Sclerotinia* attacking white clover, but found other forage legumes more susceptible.

During the spring of 1945 and again in 1946 extensive killing of Ladino clover stolons was observed in nursery and field plots at State College, Pennsylvania. Many of the stolons were affected by a watery soft rot that left 4 or 5 cm. of tissue collapsed and flaccid. Other stolons in more exposed places were dead and disintegrating as though attacked earlier. In some cases, white masses of mycelium grew on the surface of diseased stolons and later formed sclerotia. Most sclerotia matured on the outside of affected stolons although some were found inside. These usually ruptured the stolon as they enlarged and were left in the soil when the stolon disintegrated. Apothecia emerging from a sclerotium imbedded in a stolon of Ladino clover are shown in figure 1, A.

Sclerotia were black, varied from 2 to 10 mm. in diameter, and were either simple or compound. Apothecia were light brown, the disk rested on the surface of the ground or was raised a few millimeters on a stipe. Stipes supporting the apothecia were 3 to 20 mm. long depending on the depth at which sclerotia were buried. Apothecia were collected from October until snowfall—only rarely were they found in the spring. Typical apothecia growing from sclerotia are in figure 1, B. On the basis of size and morphology of sclerotia and apothecia, cultural characteristics, and pathogenicity to Ladino clover and other legumes, the organism was identified as *Sclerotinia trifoliorum*.

The present study was undertaken because of the potential destructiveness of the disease to a valuable forage crop.

MATERIALS AND METHODS

Cultures of the organism were obtained from sclerotia and ascospores. Sclerotia were surface sterilized, halved aseptically, and plated on Difco potato-dextrose agar in Petri dishes.
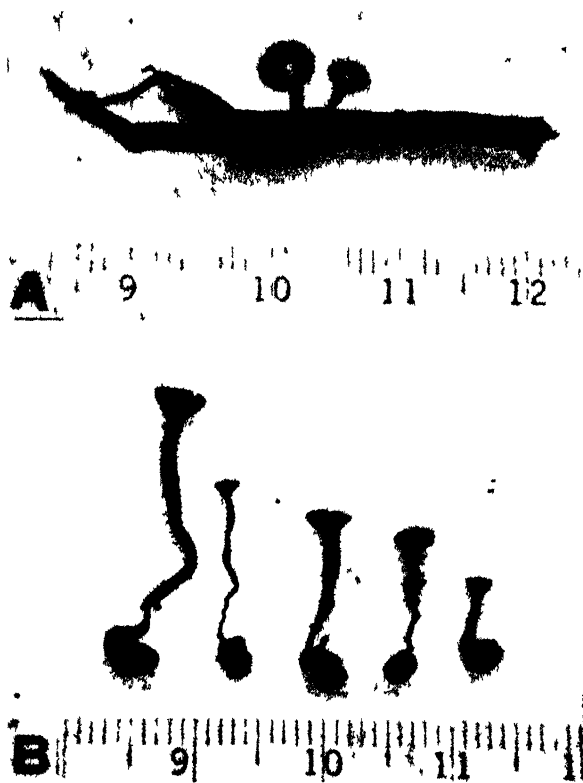
FIG. 1. A. Apothecia emerging from a sclerotium imbedded in part of a dead and disintegrating stolon of Ladino clover. B. Apothecia with stipes of different lengths. A millimeter scale is shown in both photographs.

Monoascospore cultures were obtained by a modification of Tapke's method (6). Instead of placing apothecia in small vials of alcohol as described by Tapke, an apothecium was held with a forceps just below the disk and a drop of 95 per cent ethyl alcohol was applied to the base of the stipe. By holding the discharging apothecium inside a partially covered Petri dish of agar while slowly rotating the dish, a fairly uniform distribution of ascospores was achieved. Individual spores were located with the high power lens of a dissecting microscope and transferred to a Petri dish of agar so marked that each spore could be readily located and observed for germination. If the spore germinated and was free of contaminants, it was trans-

ferred to a fresh tube of potato-dextrose agar.   In this way, more than 100
monoascospore cultures were isolated from apothecia collected in stands of
Ladino and red clover.

Infection tests were conducted with cultures obtained from sclerotia
and from ascospores.   Hosts inoculated included Ladino clover, red clover,
other species of *Trifolium, Medicago, Melilotus,* and *Lotus.*

CULTURAL CHARACTERISTICS OF ISOLATES

On Difco potato-dextrose agar, most cultures produced a white, ap-
pressed to fluffy colony with regular margins.   Growth was usually rapid,
the mycelium frequently reaching the periphery of a 90-mm. Petri dish in
5 days at 20° C.   Most cultures produced sclerotia after the hyphae reached
the edge of the dish.   Sclerotia were first noticeable as clusters of dense,
white mycelium.   These gradually enlarged, turned gray and then black.
Most cultures produced mature sclerotia in 14 to 21 days.

Some monoascospore isolates grew more slowly than others.   These either
eventually produced clumps of mycelium resembling partially developed
sclerotia or maintained a uniform mycelial growth.   The majority of cul-
tures were classified into 4 types: (1) closely appressed mycelium, no
sclerotia (2) appressed mycelium toward the center but the outer one-fourth
of the colony composed of fluffy, white mycelium; no sclerotia (3) mostly
appressed mycelium with an outer ring of fluffy mycelium containing a few
large, compound sclerotia (4) same as type 3 but with many sclerotia around
the outer edge of the colony.   There was no noticeable cultural difference
between isolates obtained from red clover or from Ladino clover.

INFECTION TECHNIC

Preliminary infection tests were conducted on mass seedings of Ladino
clover in flats of steamed soil.   After the seedlings reached the 2 or 3 leaf
stage and formed a solid cover, they were inoculated by scattering pieces
of agar inoculum on the soil among the plants.   The flats were then mois-
tened, covered with a double layer of cheesecloth and incubated on a green-
house bench at 18°–20° C.   This method resulted in death of seedlings in
patches where they had been exposed, but was found unsatisfactory because
much of the agar inoculum was rapidly overgrown by contaminants that
reduced its effectiveness.   To overcome this difficulty, cultures were grown
for 21 days on a medium composed of 2 parts wheat, 1 part oats (5).   This
time was ordinarily required for the organism to spread through the grain
and begin to form sclerotia.   The effectiveness of the fresh grain inoculum
was tested on crowns or stolons of potted plants under high humidity and at
18°–20° C.   Good infection was obtained.

EFFECT OF SCLEROTINIA ON INOCULATED LADINO CLOVER

When fresh grain inoculum was spread along a row of Ladino clover
seedlings and the inoculated plants were kept moist and at 18°–20° C., they

became infected in 3 or 4 days. Earliest visible symptoms consisted of drooping and wilting of leaves and petioles. Damaged leaves usually turned gray-green as though scalded and the infected seedlings became flaccid and collapsed shortly thereafter. Most seedlings were dead 7–10 days following inoculation.

Similarly, when grain inoculum was applied to the crowns or stolons of older plants growing in pots they were attacked as readily as seedlings but it took longer to kill them. Younger leaves and petioles were infected and collapsed first; stolon tips and lateral buds were attacked next. As the mycelium overgrew other parts of the plant, older leaves and stolon internodes were damaged or killed. Stolons infected under controlled conditions became flaccid and watersoaked like those naturally infected in the field. When handled, the stolons were readily pulled apart.

PATHOGENICITY OF MONOASCOSPORE ISOLATES TO LADINO AND RED CLOVER

Preliminary tests showed that cultures of *Sclerotinia trifoliorum* obtained from sclerotia were pathogenic to seedlings and mature plants of Ladino clover. Additional tests were therefore conducted to determine the pathogenicity of monoascospore isolates. Björling (1), Frandsen (2), and others emphasized that a sclerotium may be comprised of the mycelium of one or more biotypes of *Sclerotina*. Observation of anastomoses between hyphae indicates that heterocaryotic mycelium may develop and form sclerotia. Cultures derived from such sclerotia would therefore not represent homozygous lines of the organism. For the pathogenicity trials, 28 monoascospore cultures representing isolates from 13 apothecia and from each of the 4 cultural types were selected. After each isolate was cultured on the wheat-oats medium fresh inoculum from it was placed in contact with crowns of seedlings and older plants of Ladino clover and mature plants of red clover. In the tests with Ladino clover, 4 seedlings and 6 mature plants were inoculated with each isolate of *Sclerotinia*. In the trial with red clover, 12 mature plants were inoculated with each isolate. The inoculated plants were thoroughly watered, placed at random and incubated in a large moist chamber (4) in a greenhouse at 18°–20° C. During late spring when greenhouses became too warm, inoculated plants were incubated in a constant temperature cold room at 15° C. Plants incubated in the cold room received illumination from 150-watt Mazda lamps 12 hours each day. Depending upon position, each plant received 50–400 foot candles of light as measured by a Weston photometer.

Most monoascospore isolates from Ladino or red clover were equally pathogenic to each host. With few exceptions, seedlings of Ladino clover were more susceptible than older plants. There was some indication of differences in virulence among isolates but striking differences were probably masked because of variation in physiologic resistance among plants of each host tested. Similarly, it was not possible to distinguish slight variations in pathogenicity among monoascospore cultures from individual apothecia.
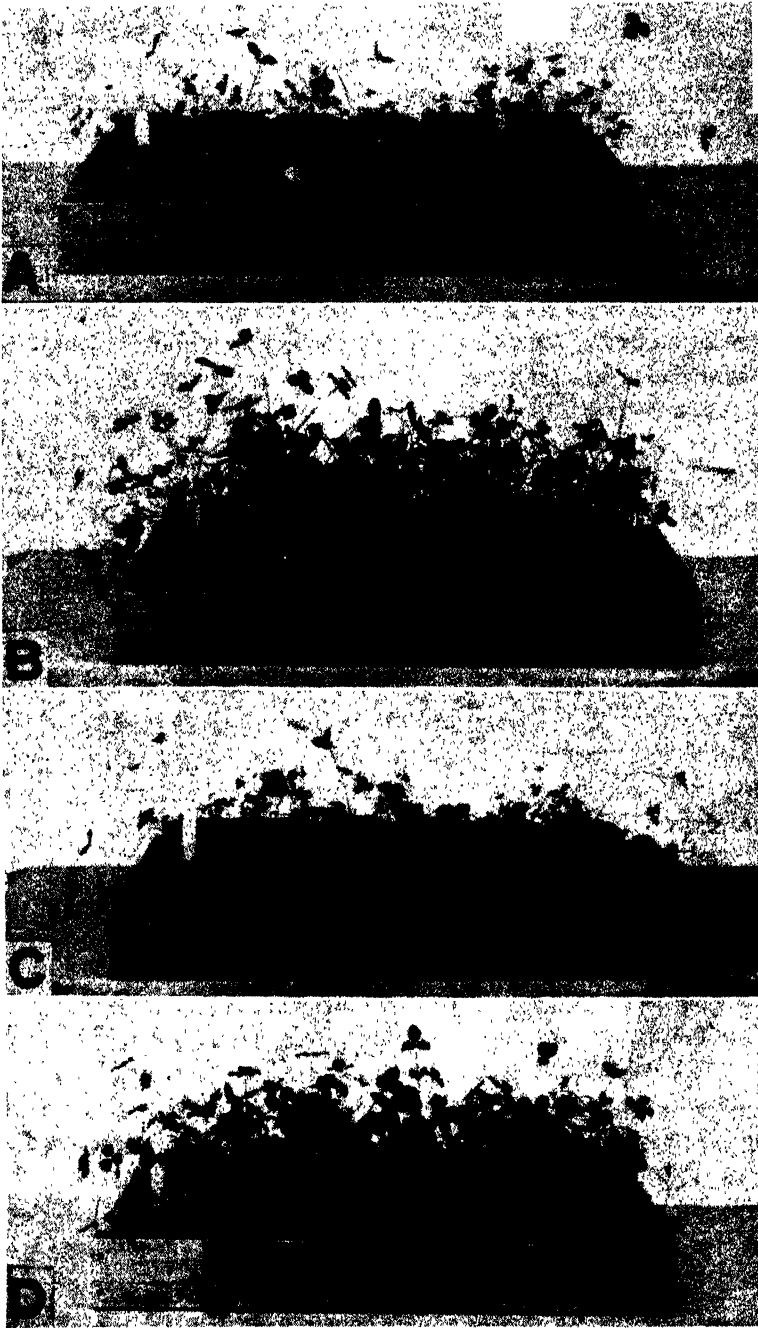
Fig. 2. Flats of 18 representative pots of Ladino clover inoculated with (A) pathogenic isolate R-1-4 from red clover, (B) less virulent isolate R-3-8 from red clover, (C) pathogenic isolate L-6-8 from Ladino clover, and (D) less virulent isolate L-1-10 from Ladino clover.

Since differences in pathogenicity of monoascospore isolates were indicated, larger numbers of plants were inoculated in a second test.

The plants were grown in 3-inch pots in a greenhouse and the stolons were clipped when they reached the edge of the pot. Four monoascospore isolates were selected: one pathogenic isolate (L-6-8) from Ladino clover and one (R-1-4) from red clover, and a less virulent isolate from each host (L-1-10 and R-3-8). Each isolate was grown in flasks of wheat-oats mixture and the inoculum was scattered over stolons of each plant. Care was taken to inoculate each plant with approximately the same amount of inoculum. Controls were not inoculated. The plants were then watered and incubated in a cold room at 15° C. The plants received illumination from 150-watt Mazda lamps 12 hours each day.

Striking differences in pathogenicity among the selected isolates (Table 1) were readily detected when the larger numbers of plants were used. Though there undoubtedly were differences in susceptibility among plants

TABLE 1.—*Comparative virulence of four isolates of Sclerotinia trifoliorum to mature plants of Ladino clover*

Infection Class	Number of plants in each infection class after inoculation with isolate				
	R-1-4	L-6-8	R-3-8	L-1-10	Control
Slight	5	9	41	23	0
Slight +	0	1	14	16	0
Moderate	12	8	14	15	0
Moderate +	11	14	2	4	0
Severe	24	21	1	2	0
Severe +	17	10	0	0	0
Dead	3	1	0	0	0
No injury	0	0	0	0	18
Totals	72	64	72	60	18

within each group inoculated, the overall damage caused by each virulent isolate was greater than that of its less virulent counterpart. This confirmed the observation of differences in virulence among monoascospore isolates tested earlier. The use of clones would perhaps reveal more subtle differences in pathogenicity and would reduce the number of plants needed for a test. The effect of pathogenic and less pathogenic isolates on plants of Ladino clover is shown in figure 2.

PATHOGENICITY TO OTHER SPECIES OF LEGUMES

Both Pape (5) and Frandsen (2) published reviews of literature concerning the host range of *Sclerotinia trifoliorum*. According to Frandsen (2) more than 80 species in 15 families are attacked by *Sclerotinia*. However, races did not appear to be restricted to certain host species since cultures from different legumes were no more infective to individuals of a selected host spectrum than were isolates from one host. Since *Trifolium repens* was listed as a species ordinarily resisting infection, it seemed desirable to test isolates from Ladino clover for their pathogenicity to other species of *Trifolium, Medicago, Melilotus,* and *Lotus.*

Three pots of seedlings and four or five mature plants of each species were inoculated. Seedlings were 6 to 10 weeks old while mature plants were either flowering or were in bud and in some cases required clipping before they were inoculated. Inoculum consisted of a composite sample of 7 pathogenic monoascospore isolates grown on the wheat-oats medium. The inoculum was distributed at the base of seedlings and around the crown of older plants; controls were not inoculated. All plants were incubated at 18°–20° C. in a greenhouse. Notes on infection were taken at intervals from 7 to 15 days following inoculation.

FIG. 3. A. *Trifolium hybridum* killed by *Sclerotinia trifoliorum*. B. *Trifolium glomeratum* severely damaged by *Sclerotinia*. In each photograph a noninoculated control is at the left.

Most species tested were susceptible to the inoculum used (Table 2). Several species of *Trifolium*, *Medicago*, and *Melilotus* not previously tested were infected. At least 3 species, *Trifolium resupinatum*, *T. fragiferum*, and *T. dubium*, were not damaged so severely as the others. *T. dubium*, in particular, appeared moderately resistant to infection with the isolates used. This suggests possible sources of resistance to the disease in species of the genus *Trifolium*. There was no appreciable difference between seedling and mature plant reaction. Occasionally differences occurred, but in most cases, these could be attributed to drying or contamination of inoculum before the organism became established on a host. The severity with which *Sclerotinia trifoliorum* attacked some species of *Trifolium* is illustrated in figure 3.

TABLE 2.—*Susceptibility of seedlings and older plants of legume species to artificial inoculation with Sclerotinia trifoliorum*

Species	Infection[a] on seedlings	Infection[a] on older plants
Trifolium repens (Ladino)	S+	S–M*
T. repens (White Dutch)	M	M
T. pratense	S+	S
T. fragiferum	M	Sl
T. hirtum	S	M*
T. carolinianum	S+	–*
T. incarnatum	S+	S–
T. resupinatum	Sl–	Sl*
T. subterraneum	S	–
T. striatum	S+	M*
T. glomeratum	Sl–	S+*
T. arvense	Sl–	S–M*
T. procumbens	Sl	S*
T. dubium	Sl	Sl+*
T. lappaceum	S+	S+*
T. agrarium	S–	S*
T. reflexum	S+	–*
T. cernuum	M+	S+*
T hybridum	–	S+
T alexandrinum	S+	S+
Medicago arabica	S+	S–M*
M. obscura	Sl–	S*
M. lupulina	Sl+	S+
M. hispida	S+	Sl
M. minima	M	M*
M. sativa	–	S+
Melilotus alba	S+	S+
M. indica	S+	S–*
M. suaveolens	S	S+*
M. officinalis	S+	S+
Lotus corniculatus	Sl–	S

[a] Sl = slight infection.
M = moderate infection.
S = severe infection.
(+) = greater infection; (–) = less infection.
* Denotes species not previously listed as susceptible to *Sclerotinia trifoliorum*.

DISCUSSION

Ladino clover is rapidly becoming one of the most important pasture plants in areas of the United States where it is adapted. As it becomes more widely grown, diseases affecting it appear more frequently and with greater destructiveness, particularly in localities where large acreages are found. *Sclerotinia trifoliorum* is one of the pathogens potentially capable of causing severe damage to a stand. Because the fungus attacks stolons at low temperatures, much of the damage observed in early spring may be attributed to winter-injury.

Observations indicate *Sclerotinia* is probably not the sole cause of winter-injury to this crop. If conditions are favorable, however, there is every indication that the disease may play a major role in loss of stands.

Results of studies with red clover by European workers indicate that the disease can probably be best controlled by breeding resistant varieties.

Should adequate sources of resistance not be found in *Trifolium repens*, there is evidence that some measure of resistance exists among other species of *Trifolium*. This might prove useful if species hybridization in the genus *Trifolium* can be accomplished.

SUMMARY

Sclerotinia trifoliorum was observed attacking Ladino clover.

Monoascospore isolates yielded four cultural types that varied from strictly mycelial types to forms that produced numerous sclerotia. Pathogenicity of monoascospore isolates was established on Ladino and red clover. Differences in pathogenicity of several monoascospore cultures were determined with a number of plants of Ladino clover.

Infected stolons of Ladino clover became flaccid and watersoaked, and death resulted after a severe attack. Seedlings collapsed and died 7 to 10 days following inoculation.

Though a composite sample of the organism was pathogenic to a number of species of *Trifolium*, *Medicago*, and *Melilotus*, several species of *Trifolium* resisted infection.

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PYTHIUM ROOT ROT OF AROIDS AND EASTER LILIES

W. B. TISDALE AND GEORGE D. RUEHLE

Chinese Evergreen (*Aglaonema simplex* Blume) became a commercial crop in Florida about 1934, at which time there were about two acres planted in the State. During the following eight years there was an increase to approximately 15 acres. This in turn has been followed by rapid decline due to Pythium root-rot.

During the period of expansion, Chinese Evergreen was produced in outdoor beds under slat sheds, which involves a high overhead investment. For such an investment to yield profitable returns, it was necessary in the early years to grow successive crops of the Chinese Evergreen. As demand warranted, other ornamentals, mostly aroids, were produced under the sheds, in rotation with Chinese Evergreen. The soils used for these crops were mostly low and sandy, but they were maintained in a high state of fertility by the addition of muck, compost, and commercial fertilizers, and a mulch of leaves or other vegetable matter was placed on the soil surface after the plants were set.

Prior to the war, most of the propagating material was imported from China. The imported canes were cut into sections bearing one or two "eyes" and started in greenhouses or planted directly in the beds under the sheds. This was usually done in April or May, and some of the resulting plants were marketed by the end of the same year. During the war when the canes could not be imported, growers began to propagate the plants by bud cuttings. This method of propagation produces marketable plants in a shorter period than propagation by cane cuttings, but it does not reduce the loss from the root-rot to any appreciable extent.

The first trouble with Pythium root-rot of Chinese Evergreen in Orange County was reported in 1943.[1] Since that time the disease has been observed in Chinese Evergreen, other aroids, and Easter lily in several localities of the State. When the first inspection of nurseries was made in Orange County, affected plants occurred in areas of various sizes, but within a year or two the entire area in some nurseries had become involved.

First sign of the trouble on above-ground parts of the plants is stunting. This is followed by drooping of the leaves, yellowing, and death of the lowest ones. Finally, some of the stems rot off at the ground level and the plants drop over, but decay of the stems apparently does not continue. Early symptoms of the disease on the roots are manifest as light-brown, water-soaked lesions of various lengths, occurring on any part of the root. In later stages the diseased tissue turns brown and disintegrates so that most of the diseased roots remain in the soil when the plants are

[1] Tisdale, W. B. Root rot of aroids. U. S. Dept. Agr., Plant Dis. Rptr. 27: 306–308. 1943.

removed. In the final stages all roots have rotted and nearly all leaves up to the bud are dead. Many mulched plants develop roots above the soil level and these become infected on coming in contact with the soil.

Several other aroids planted under sheds with Chinese Evergreens had similar disease symptoms on the roots, but in no case was the damage as severe as that in Chinese Evergreen. The plants affected were *Dieffenbachia picta* Schott., *Nephthytis Afzelii* Schott., *Philodendron cordatum* Kunth., and *Scindapsus aureus* Engler.

In 1944 a grower in Dade County reported a serious condition of Easter lilies in his planting. He had noticed the disease in milder form the previous year, and bulbs from that crop were planted on the same land, and on additional acreage in 1944. The soil was of the Rockdale series, and very different from that in which the Chinese Evergreen and other aroids are produced. When inspected in March of that year, practically every plant in fields planted to Easter lilies the previous year showed advanced stages of the disease, and the condition of plants in other fields from the same lot of bulbs was little better. The plants were severely stunted, the lowest leaves were dead, and the other leaves were yellow with dead tips. Some plants were dead except for a few bud leaves. This condition may have been caused in part by virus diseases, but the plants were in such advanced stages of decline that diagnosis was impossible.

Root decay was extensive in all plants examined, and many roots were dead. New roots had developed on some stems at about the ground level and these had dead tips or brown lesions on other parts. Also, the outer scales of many bulbs were dead or showed brown lesions of various sizes. Some bulbs of the 1944 crop were saved and planted later in the year, and they failed completely. Since 1945, commercial growing of Easter lilies on the Rockdale series of soils in Dade County has been practically discontinued because of the losses caused by Pythium root rot.

Several fungi were obtained consistently in cultures from all the host plants, but none of them, except *Pythium splendens* Braun,[2] produced any symptoms of disease in inoculation experiments. Cultures of the *Pythium* isolated from all the aroids listed above and Easter lily have produced a root-rot typical of that developing in them in the nurseries, and cultural characters and cross inoculations have shown that the causal organism is the same for all crops, and is widely distributed in Florida. *Pythium splendens* has been reported to attack a wide range of host plants,[3] but none of the ones specified in this article was included in the list.

When rooted cuttings of Chinese Evergreen and young Easter lily plants were set in artificially inoculated soil held at moderate temperatures, root lesions developed within 10 days and the root systems were almost completely destroyed in 21 days (Fig. 1 and 2). In all tests the disease developed most rapidly at moderate temperatures, and least rapidly at

[2] Determination made by John T. Middleton.
[3] Middleton, John T., The taxonomy, host range and geographic distribution of the genus Pythium. Mem. Torrey Bot. Club 20: 1–171. 1943.

FIG. 1. Pythium root rot of Chinese Evergreen. Healthy plant at left set in sterilized soil and diseased plant after 21 days in soil artificially inoculated with *Pythium splendens*.

high temperatures This coincides with observations made in the nurseries.

Activity of the fungus and its distribution in the nurseries suggests general sterilization of the soil for control. A preliminary test made in a

FIG. 2. Pythium root rot of Easter lily. Healthy plant at left set in sterilized soil and diseased plant (right) after 21 days in soil artificially inoculated with *Pythium splendens*.

nursery in which the soil was treated with one-half gallon of 1–50 formaldehyde solution per square foot gave about 85 per cent good plants that had only a few root lesions when they were of marketable size. However, the fact that the beds became reinfested before the plants reached marketable size indicates that the walkways between beds and bed wall boards, must be treated to prevent reinfestation in a short time.

Several of the new fungicides were tested in potted soil to ascertain whether they would eradicate the fungus from the soil without injuring the plants. Tersan (tetramethyl thiuram disulfide) and Fermate (ferric dimethyl dithiocarbamate) were the only materials tested that showed promise for this purpose. After the plants were set in infested soil enough of the solution containing 1 or 2 gm. of the chemical to 1 liter of water was added to wet the soil throughout the pot. Neither of these concentrations appeared to injure the roots, and both prevented infection. However, in outdoor tests where 1 pint of the solution per square foot was dug into the soil immediately before setting the plants and was followed with the same volume at 30-day intervals, applied on the soil surface with a sprinkling can, no appreciable benefit was obtained. Some growers have resorted to propagating Chinese Evergreen on benches in the greenhouse, using steam-sterilized soil. When clean planting stock is used, the plants have remained free of Pythium root-rot, but this has necessitated a great reduction in the number of plants propagated.

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The Rôle of Thiamin and Certain Related Compounds in Pigment Production by Corynebacterium michiganense (Erw. Smith) Jensen.—Variation in pigmentation has been described for a number of plant pathogenic as well as for many other bacterial species. Bryan,[1, 2] for example, has isolated white and pink variants of the usual yellow form of *Corynebacterium michiganense* (Erw. Smith) Jensen. More recently Ark[3] with the use of acenaphthene-saturated broth induced white mutants in this species. A correlation was found to exist[4] between pigmentation and virulence. The yellow form was found to be the most virulent for the tomato, while the pink form was far less pathogenic. Despite the fact that considerable work has been done on pigmentation, very little is known about the physiological mechanism involved in the production of these substances by bacteria.

The findings reported here are the result of observations made on two isolates of *Corynebacterium michiganense* that had been kept as stock cultures for a number of years. In their cultural, staining, and disease-producing properties both were typical of the species. It was observed that when these isolates were cultivated on certain media such as (Difco) dextrose agar growth was excellent at 25° C. but there was not the slightest indication of the presence of the characteristic yellow pigment. When the dextrose agar was supplemented, however, with one per cent to two per cent (Difco) yeast extract the yellow pigment was produced. These results demonstrated, therefore, that pigmentation in this instance was dependent upon some factor or factors present in the yeast extract. As a result of this finding certain vitamins of the "B" complex which included thiamin HCl, pyridoxine, niacin, riboflavin, Ca pantothenate, inositol, and para aminobenzoic acid were tested in concentrations ranging from 100 mgs. to 0.01 mg. per liter of media. All were sterilized by filtration. Of the substances tested only thiamin HCl was capable of replacing the yeast extract in the dextrose agar. Excellent yellow pigmentation was shown by the bacteria when concentrations of this compound ranging from 100 mg. to 1 mg. per liter of media were used. A cream colored growth was produced at a concentration of 0.1 mg. of thiamin HCl per liter. The thiazole (4-methyl-5-β-hydroxyethyl thiazole) portion of the thiamin molecule was capable of replacing the thiamin, while the pyrimidine fraction (2-methyl-5-ethoxymethyl-6-aminopyrimidine) was without effect. A sample of γhydroxypropyl thiazole, however, was inactive. Cocarboxylase, on the

[1] Bryan, Mary K. An albino strain of *Aplanobacter michiganense*. (Abstr.) Phytopath. 20: 141. 1930.

[2] Bryan, Mary K. Color variations in *Aplanobacter michiganense*. Phytopath. 21: 559. 1931.

[3] Ark, P. A. Mutation in certain phytopathogenic bacteria induced by acenaphthene. Jour. Bact. 51: 699–701. 1946.

[4] Fawcett, Edna H., and Mary K. Bryan. Color in relation to virulence in *Aplanobacter michiganense*. Phytopath. 24: 308–309. 1934.

other hand, was effective in the same concentrations as was thiamin and thiazole. A white mutant of this organism did not respond to thiamin. The physiological action of these compounds in pigment production is being investigated.—ARMIN C. BRAUN, Department of Animal and Plant Pathology, The Rockefeller Institute for Medical Research, Princeton, New Jersey.

A New Method of Evaluating Fungicides.—Most of the laboratory methods of evaluating fungicides are based on the ability of the fungicides

TABLE 1.—*Relation of concentration and exposure time to effectiveness of five fungicides against Fusarium oxysporum (Schl.) var. gladioli Massey*

Fungicide and concentration[a]	Exposure time and fungus growth[b]			
	15 min.	30 min.	1 hr.	2 hr.
Dow 9B (zinc trichlorophenate)				
1.00 per cent	−	−	−	−
0.50	+	+	−	−
0.25	+	+	+	−
Puraturf 177 (phenyl mercuri triethanol ammonium lactate)				
2.00 per cent	−	−	−	−
1.00	+	−	−	−
0.50	+	+	−	−
0.25	+	+	+	+
New Improved Ceresan (ethyl mercury phosphate)				
0.047 per cent	−	−	−	−
0.031	+	−	−	−
0.016	+	+	−	−
0.008	+	+	−	−
0.004	+	+	+	+
Puratized Agricultural Spray (phenyl mercuri triethanol ammonium lactate)				
0.06 per cent	−	−	−	−
0.04	+	+	−	−
0.03	+	+	+	−
Mercuric chloride				
0.010 per cent	−	−	−	−
0.006	+	−	−	−
0.005	+	+	−	−
0.004	+	+	+	−

[a] Concentration was based on the commercial products and not on active ingredients.
[b] + indicates growth of the fungus; − indicates no growth.

to inhibit spore germination. Since none of the methods commonly employed is especially suited for use with Fusarium, the effectiveness of various fungicides for control of such diseases as Fusarium rot of gladiolus has been determined entirely by field tests. With the introduction of so many new fungicidal materials it is becoming increasingly difficult to test them on a field basis. A simple laboratory test has been developed to save time in screening fungicides effective against the gladiolus Fusaria.

Several sterile pieces of No. 8 cotton thread 3 to 4 inches long are laid

on the surface of potato-dextrose agar in a Petri dish (Fig. 1, A).   The
fungus is planted in the plate and allowed to grow until the entire surface
of the agar is covered by the fungus.   The pieces of thread are then re-
moved from the culture plate with a sterile forceps and cut into pieces
about ¼ inch long with sterile scissors.   These small pieces are collected in
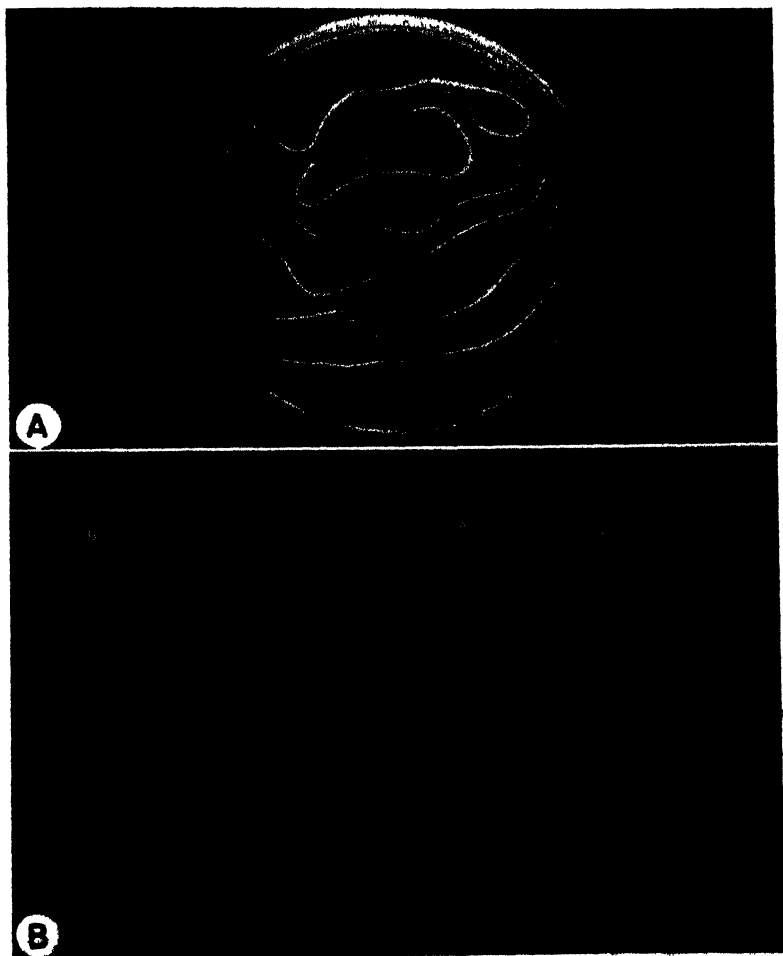
FIG. 1. A. Fusarium growing over cotton threads on surface of agar plate. B.
Growth of Fusarium from treated threads 5 days after plating; left to right, upper row:
water, Parzate, Puraturf 177; lower row: Dow 9B, Lysol, New Improved Ceresan.
Starting at upper right sector in each plate and reading clockwise, treatments were 2
hours, 1 hour, 30 minutes, and 15 minutes.

a sterile Petri dish from which they are removed with a sterile forceps and
placed in the fungicide preparation.
   As a preliminary test, pieces of the fungus-infested thread are rolled
in a small quantity of the dry fungicide until they are well coated and
then planted in potato-dextrose agar plates.   The plates are kept at room

temperature (approx. 22° C.) for 7 days and examined for growth of the fungus. Materials which do not completely inhibit growth of the fungus are not tested further.

Several concentrations of the effective materials are made up for further testing. The small pieces of fungus-infested thread are immersed in these prepared solutions and allowed to remain for predetermined lengths of time. They are then removed, touched momentarily to a piece of sterile filter paper to remove the excess solution, and planted in an agar plate. Each series is run in duplicate. The plates are kept at room temperature for 7 days and examined for growth of the fungus.

A sample of the results of one test in which this method was used is shown in table 1. These data show that two factors are involved in inhibiting growth of the fungus. They are concentration of the fungicide and length of exposure to the fungicide. Even though a material is not fungicidal when used at low concentrations or with short exposures, some fungistatic action is evident. As shown in figure 1, B, fungistatic action increases directly with the length of exposure until the fungicidal point is reached.

The method should prove useful in testing fungicides against any fungus that grows well on laboratory culture media. It saves time in determining which materials merit further trial on a field basis and at the same time gives some idea as to the concentrations and treating times that will be necessary to be effective.—J. L. FORSBERG, Section of Applied Botany and Plant Pathology, Illinois Natural History Survey, Urbana, Illinois.

Rhizoctonia Neck and Bulb Rot of Iris in the Pacific Northwest.[1]—For the past two years a neck and bulb rot of the Wedgewood variety of bulbous iris (*Iris tingitana* hybrid Boiss. & Reut.) has been observed in the Puyallup and Mt. Vernon bulb growing areas in Washington. This rot is present in addition to the more common crown rot caused by *Sclerotium delphinii* Welch and apparently has been confused with it. The symptoms of crown rot (*Sclerotium*) differ from the symptoms of the neck and bulb rot (*Rhizoctonia*). In crown rot, numerous small, dark reddish-brown sclerotia are associated with a rot produced on tissue at the base of the leaves and flower stalks. This crown rot causes the leaves to die back from the tips and the tops to fall over. In the case of the neck and bulb rot, however, a soft, light brown rot at the neck of the bulb results in the death of all above ground parts without any die-back of the leaf tips (Fig. 1). In severe cases of neck and bulb rot dark brown lesions occur on the outer scales, and the bulbs may be almost completely rotted away. These symptoms correspond to those reported as caused by *Rhizoctonia* on tulips by MacLean.[2] No sclerotia were found.

[1] Published as Scientific Paper No. 795, College of Agriculture and Agricultural Experiment Stations, Institute of Agricultural Sciences, State College of Washington, Pullman, Wash.

[2] MacLean, Neil Allan. Rhizoctonia rot of tulips in the Pacific Northwest. Phytopath. **38**: 156–157. 1948.

FIG. 1 Symptoms of *Rhizoctonia solani* injury on Wedgewood iris. × ½.

Isolations made on 2 per cent potato-dextrose agar from decayed tissue developed a light brown, slow growing, mycelium with the typical right-angled branching and constrictions of *Rhizoctonia*. Hyphal strands measured 5.5–8 μ in diameter The causal agent is believed to be *Rhizoctonia solani* Kuehn —CLARENCE II SONDERMAN and NEIL ALLAN MACLEAN, State College of Washington, Pullman, Wash.

SPRAYING EXPERIMENTS TO CONTROL CITRUS CANKER[1]

L I A N G   H W A N G

Citrus canker, caused by *Pseudomonas citri* Hasse, once came into prominence in Florida for a short time, but was eradicated from all commercial orchards there and in the other Gulf States of the United States. Fawcett (1) has recorded the known distribution of the disease in the Orient and other countries of the world, and Tanaka (6) has discussed the history of the canker in Japan, but the writer wishes to report briefly about the disease in South China. According to Lin (4), citrus canker was rather prevalent and destructive to sweet orange (*Citrus sinensis*) and mandarin orange (*C. nobilis*) in Foochow and Changchow of Fukien Province, but it was very slight on sweet orange in Gong Hsien and rather severe on lemon (*C. limonia*) in Nan-feng of Kiangsi Province. The writer (2) has reported that the disease is widely distributed in Kwangsi Province: it has been most destructive and prevalent on the pummelo group (*C. grandis*) in 8 of the 22 districts. Sweet orange and mandarin orange groups are less severely infected, while the tangerine group (*C. nobilis* var. *deliciosa*) and lemon are only slightly infected.

Since this disease is well established in most citrus growing areas in China, neither exclusion nor eradication are possible. An effective control measure is very necessary.

EXPERIMENTAL METHODS

A 4–6–50 Bordeaux mixture, alone or with materials to control insect pests, and a 1–500 lime solution, alone or with a sticker, were tested for their fungicidal efficacy in citrus orchards at the Kwangsi Agricultural Experiment Station, Sha-tang, Liu-chow, Kwangsi.

The Bordeaux mixture was used alone in 1940. In 1941, in order to control chewing insects, 0.5 lb. lead arsenate was added to each 50 gal. Bordeaux. The next two years, 1942 and 1943, a modified Bordeaux-oil emulsion was used so that wooly aphis and scale insects as well as the citrus canker bacteria might be controlled. The oil emulsion was prepared from 2 gal. vegetable oil (tea oil preferred), 2 lb. pure soap, and 1 gal. water. The mixture was boiled a few minutes and kept as a stock supply. Three parts by volume were added to 200 parts of Bordeaux mixture and thoroughly mixed at spraying time.

The lime solution was used alone in 1940. A sticker, 3 lb. alum to 50 gal. spray solution, was added in 1941. The lime solution sprays were somewhat unsatisfactory and were not used in the next two years.

Three spray schedules were followed each year (Table 1). Schedule I was arranged so that sprays were applied when new flushes of foliage were produced by the trees. Schedules II and III were arranged so that spraying was done when temperatures were suitable for infection by the canker bacteria, but there were longer intervals between spray applications in Schedule III than in Schedule II.

Newly unfolded young leaves of citrus are most susceptible to the canker bacteria and protection of new foliage flushes is of prime importance in controlling the disease. According to S. L. Hsiong, new flushes appear about five times a year at Liu-chow: early February, late March, mid-May, mid-July, and September. New flushes depend upon frequency and abundance of precipitation and vary somewhat each year.

TABLE 1.—*Spray schedules for the control of citrus canker in Kwangsi Province, China, for 1940 through 1943*

Year	Schedule	Spraying dates
1940	I	June 15, July 16, Aug. 16
	II	June 6, Aug. 7, Aug. 31
	III	Aug. 7, Aug. 16, Aug. 31
1941	I	Apr. 17, July 17, Oct. 17
	II	Apr. 30, June 16, July 31
	III	May 3, July 1, Sept. 1
1942	I	Apr. 5, July 2, Oct. 1
	II	Apr. 16, May 28, July 16
	III	May 2, July 2, Sept. 7
1943	I	Mar. 17, May 30, July 30, Sept. 30
	II	Apr. 1, May 17, July 3, Aug. 17, Sept. 30
	III	Apr. 1, June 3, Aug. 2, Oct. 2

Young citrus leaves may be infected by the bacteria when temperatures range from 20° to 35° C. and free moisture is on the leaf surfaces for 20 minutes or longer (5). According to K. C Chu's "Weather of China," atmospheric temperatures in the important citrus growing districts of Kwangsi Province were 19°–20.6° C. in April and 20.5°–23.7° C. in October in 1935. Upon checking weather records for 12 districts of the Province, the writer found that average temperatures during the 3-year period 1938–1940 were 19°–22 3° C. in April and 15.7°–20.2° C. in November. The precipitation was greatest between May and August and was least in December, January, and February.

In 1940 three orchards of 5-year-old trees were sprayed. They were *Citrus nobilis* var. *Poonensis* Hayada, *C. nobilis* var. *Unshiu* Swingle, and the very susceptible *C. grandis* Osbeck var. *Sha-tien*. In 1941 the same three varieties of citrus were sprayed, but a fourth variety, *C. sinensis* var. *Heung-shue-cheng* was included. Only trees of the very susceptible pummello group, *C. grandis* var. *Sha-tien*, were sprayed in 1942 and 1943. In the last year, 1943, large numbers of 2-year-old seedlings were sprayed: more than 100 seedlings of sour orange (*C. aurantium* var. *Kiang-tsin*), about the same number of Sunki seedlings (*C. nobilis* var. *Sunki*), and as many Mou-yu seedlings (*C. grandis* var. *Mou-yu*).

All trees were naturally infected with the citrus canker bacteria. Disease notes were recorded before spraying began and again two months after the final spraying in each schedule. Four disease classes were set up and plus and minus signs were used to designate variability within a class: Trace indicated an average of only one lesion for each ten leaves, Light indicated an average of only one lesion for each five leaves, Moderate signified an average of two lesions on each leaf and a few lesions on twigs and fruits, and Severe signified an average of five lesions on each leaf and numerous lesions on twigs and fruits.

RELATIVE EFFICACY OF BORDEAUX MIXTURE AND LIME SOLUTION

There was considerable natural infection in the orchards in 1940, and Bordeaux mixture was somewhat more satisfactory than lime solution in re-

TABLE 2.—*The effect of Bordeaux mixture (4-6-50) and lime solution (1-500) on citrus canker in three varieties of citrus in 1940*

Species and variety of citrus	Fungicide	Spray schedule[a]	Infection on trees	
			Before spraying	After spraying
C. nobilis Poonensis	Bordeaux	I	Moderate −	Trace −
		II	Moderate +	Trace −
		III	Moderate	None
	Lime solution	I	Moderate	None
		II	Moderate −	None
		III	Severe	Trace −
C. nobilis Unshiu	Bordeaux	I	Trace +	None
		II	Trace	None
		III	Light +	None
	Lime solution	I	Trace +	Light −
		II	Trace +	Light +
		III	Light +	Trace
C. grandis Sha tien	Bordeaux	I	Moderate +	None
		II	Moderate +	None
		III	Moderate +	None
	Lime solution	I	Moderate +	Trace −
		II	Moderate −	Trace −
		III	Moderate	Trace

[a] Dates for each spray schedule are in table 1.

ducing the amount of bacterial canker infection (Table 2). In spite of the fact that Lee (3) suggested that 1–500 lime solution was the cheapest and most effective spray in orchard practice, the results reported here indicate that Bordeaux mixture is to be preferred to lime solution. In 1941 there was relatively little natural infection in the orchards and the advantages of spraying to control citrus canker bacteria were not evident.

In the absence of insects in the orchards, Bordeaux mixture alone was satisfactory; when chewing insects were present the addition of lead arsenate insecticide proved to be good orchard practice and was not injurious to citrus foliage.

TABLE 3.—*The effect of Bordeaux-oil emulsion on citrus canker in Citrus grandis var. Sha-tien in 1942 and 1943*

Spray schedule	Infection* on trees in			
	1942		1943	
	Before spray	After spray	Before spray	After spray
I	Light	Trace	Trace	None
II	Light +	Light +	Trace +	None
III	Light −	Trace −	Light −	None
Check	Light −	Light	Trace	Light

* Results are averages for 8 trees in each schedule and in check plots.

THE EFFICACY OF BORDEAUX-OIL EMULSION

This spray, applied to trees of the very susceptible *Citrus grandis* var. *Sha-tien*, reduced bacterial infection when applied according to Schedules I and III in 1942 and when applied according to all three schedules in 1943. There was no reduction of infection when it was applied according to Schedule II in 1942 (Table 3). It is not known why Schedule II was not effective in 1942. It may have been because the final spray was applied on July 16 whereas the final spray in Schedule I was made on October 1 and that of Schedule III was made on September 7 in that year.

Seedling stocks were more variable with respect to the infection they carried after having been sprayed with the Bordeaux-oil emulsion. Since rather large numbers of seedlings were in each plot, the percentages of seedlings in each disease class were recorded (Table 4). The spray effectively reduced infection in the sour orange seedlings, but failed to do so in the Sunki and Mou-yu seedlings.

TABLE 4.—*The effect of Bordeaux-oil emulsion spray on citrus canker in seedling stocks in 1943*

Seedlings of citrus		Spray schedule	Infection before spraying	Percentage of trees in each disease class after spraying				
Kind	Number			None	Trace	Light	Moderate	Dead*
Sour orange	46	I	Tr to Lt	61	26	0	0	13
	31	II	do	87	10	0	0	3
	31	III	0 to Lt	78	23	0	0	0
	30	Check	Tr	36	53	3	0	7
Sunki	47	I	Tr	0	34	58	0	9
	32	II	Tr	0	50	34	3	13
	32	III	Tr	0	50	41	6	3
	32	Check	Tr	0	56	34	9	0
Mou-yu	36	I	Tr	0	67	25	0	8
	36	II	0 to Tr	0	64	25	6	6
	36	III	Tr	0	75	17	0	9
	36	Check	Tr	0	50	19	6	25

* Death was due to causes other than bacterial canker.

CONCLUSIONS AND SUMMARY

In three of four years the incidence of citrus canker in orchards of 5-year-old trees in Kwangsi Province was reduced by spraying with Bordeaux mixture or with Bordeaux-oil emulsion. Spray schedules set up according to the appearance of new foliage flushes or according to the temperatures favorable for the canker bacteria were satisfactory if they began early in the growing season and extended to late September or early October. Thorough spraying during the growing season not only protected citrus foliage from infection but greatly reduced the amount of infection that had been established on the trees prior to the first spray.

Because wooly aphis and scale insects are prevalent in citrus orchards of Kwangsi Province, the Bordeaux-oil emulsion probably will be the most satisfactory and economical spray material for general orchard practice.

In a single season the Bordeaux-oil emulsion effectively controlled citrus canker in sour orange seedling stocks, but it failed to control the disease in stocks of the very susceptible Sunki and Mou-yu seedlings. At present it is not possible to recommend sprays for nursery practice.

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THE CONVERSION OF STRAINS OF ALCALIGENES RADIOBACTER AND PHYTOMONAS TUMEFACIENS IN THE "S" PHASE TO THE "M" PHASE OF THE HETEROLOGOUS SPECIES

MADELINE F. COLEMAN AND JAMES J. REID

The cultural and morphological similarity of *Phytomonas tumefaciens* and *Alcaligenes radiobacter* has been recognized for many years. Patel (8) noted that the two organisms were similar in their morphological and physiological characteristics and could be separated only by pathogenicity. Similarity between certain non-pathogenic organisms and the crown-gall producing organism has been reported by Sagen, Wright, and Riker (11). Morphological and physiological studies continued by Riker et al (9) were conducted in an attempt to obtain cultural differences by growth in various media. Serological studies showed no cross agglutination between species under the conditions of their experiments. No serological relationship in the Dawson "M" phase[1] was observed and reported by us (1) in cross agglutination tests. Sagen, Riker, and Baldwin (10) reported a further study of the physiological characteristics of *Phytomonas tumefaciens, Phytomonas rhizogenes,* and *Alcaligenes radiobacter* with emphasis on their nitrogen and carbon metabolism. No separation was effected by this study. The attempts of Hendrickson, Baldwin, and Riker (7) to induce variation by repeated transfer on different artificial media met with failure. Variations in the physiological behavior of the crown-gall organism were obtained by plant passage. The authors were unable to generalize on physiological characteristics but found that virulent cultures of crown-gall bacteria secured in plant passage experiments gave *radiobacter* reaction on laboratory media. This would seem to indicate that, by laboratory procedures and plant passage, cultures which were doubtless originally crown-gall organisms had lost their pathogenicity and thus resembled *A. radiobacter* or had acquired some of the physiological characteristics of *A. radiobacter* without losing their pathogenicity. The authors suggested that such changes might occur in which the non-pathogenic crown-gall could be induced to assume the physiological characters of *A. radiobacter* or that if pathogenic crown-gall cultures which are physiologically similar to *A. radiobacter* were to lose their pathogenicity, *Phytomonas tumefaciens* might be converted to resemble *A. radiobacter.* They believe that in view of the results obtained in their study it is possible to bring about a transference of *Phytomonas tumefaciens* into an organism possessing the important characters of the "so-called" *Alcaligenes radiobacter.*

In general, the results of these extensive studies and others not cited

[1] Designation used here follows terminology suggested in (4).

here serve to emphasize the close morphological and physiological relationship between the crown-gall and *radiobacter* organisms. In an endeavor to discover the reasons for their close similarity, it seemed feasible to investigate the serological relationship of *Alcaligenes radiobacter* and *Phytomonas tumefaciens* along methods described by Dawson (2, 3) and Dawson and Sia (5) in which their serological techniques in the transformation of pneumococcal types could be applied. In a previous paper (1) the results of an investigation of the serological relationship of *A. radiobacter* and *P. tumefaciens* with the use of agglutination and agglutinin absorption techniques have been reported. This present paper deals with a continuation of that study in which the conversion of the same strains of *Alcaligenes radiobacter* and *Phytomonas tumefaciens* in the Dawson "M" and "S" phases to the heterologous species is described.

<center>METHODS</center>

Cultures used. Cultures representing both the "M" and "S" phases of three strains of *Alcaligenes radiobacter*, Ri-Ia, R3, and R3scI, and the "M" and "S" phases of two strains of *Phytomonas tumefaciens*, ScT5fffI and ScA-I, were used in this study.[2] The history of these strains and the methods used in deriving the "S" phase variants have been discussed in a previous publication (1).

Media employed. Yeast extract-mannitol-mineral salts broth or agar, considered the standard media for organisms of this mucoid type, were used for cultivation in the "M" phase. For decapsulation studies and maintenance of the Dawson "S" phase cells, the mannitol content was reduced to 0.5 per cent, in the presence of 10 per cent homologous "M" antiserum. The omission of mannitol in the *in vitro* experiments in encapsulating "S" phase cells to the "M" phase of the heterologous species produced quicker results.

Serological techniques. Methods used in animal immunization and agglutination and agglutinin absorption techniques employed have been described (1). Macroscopic agglutination tests were run routinely to check "M" and "S" phases of the cultures whenever used throughout this study.

<center>*In Vitro Experiments*</center>

The method used to encapsulate *Phytomonas tumefaciens* in the "S" phase with the *Alcaligenes radiobacter* "M" phase capsule and to place the *Phytomonas tumefaciens* "M" phase capsule on the "S" phase *Alcaligenes radiobacter* was essentially the one described by Dawson (2, 3) and Dawson and Sia (5) for the conversion of the pneumococci.

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[2] The cultures used in this study were obtained from the stock culture collection of the Division of Bacteriology, New York State Agricultural Experiment Station, Geneva, N. Y., through the courtesy of Dr. A. W. Hofer. These strains have been employed by a number of research workers in the fields of soil bacteriology and plant pathology. The strains of *P. tumefaciens* used were ScT5fffI and ScA-I. These are single-cell isolates made at the University of Wisconsin, known as typical of their species. *A. radiobacter* R3 was isolated by D. B. Fred of the University of Wisconsin in 1927. R3scI is a single-cell isolation from culture R3.

Capsular material was prepared by growing the "M" phases of the strains under investigation on standard yeast-mannitol-mineral salts agar in large Blake bottles. After 72 hours, the cultures were Gram stained and removed by washing with sterile saline and then filtered aseptically through sterile cotton into sterile vaccine bottles. Enough of each suspension was removed to use for checking its Dawson "M" phase by macroscopic agglutination tests against "M" and "S" phase antisera. The remainder was then heated in a water bath for 30 minutes at 60° C., with gentle agitation. The heat-killed vaccines were kept at room temperature for one week and tested daily for sterility by cultivation in yeast-mannitol-mineral salts broth and by streaking on plates of the same medium. The vaccines were then stored at 4° C. and sterility tests made whenever used again.

Encapsulation of the "S" phases of the strains under investigation was next attempted. Several alternate conditions were set up in the *in vitro* experiments which varied in the following manner:

1. Difference in size of inoculum of the organism to be encapsulated.
2. Difference in amount of the desired heterologous "M" phase, heat-killed vaccine.
3. Difference in amount and type of antisera.
4. Difference in mannitol content of standard yeast-mannitol-mineral salts broth or agar.

The procedure which proved successful consisted of transferring one drop of a 10^{-6} dilution of an actively growing culture in the "S" phase to 1 ml. of yeast extract-mineral salts broth to which was added 1.0 ml. of heat-killed vaccine of the desired "M" phase of the heterologous organism and 0.1 ml. of antisera prepared against the "S" phase of the homologous strain. This was done in triplicate for each strain.

These cultures were kept at room temperature and transfers were made every 24, 48, and 72 hours, by streaking and making.pin point colonies on yeast-mannitol-mineral salts agar plates. Observations of these plates were made every 24, 48, and 72 hours for typical mucoid colonies, and any suspected "M" phase colony was transferred to yeast-mannitol-mineral salts agar slants. After Gram staining, antigenic suspensions were made from 48-hour cultures to test against the heterologous and homologous "M" and "S" phase antisera. Antisera controls were run at the same time.

In Vivo Conversion

Plant Inoculations. According to the methods described by Smith and Townsend (12) and Hendrickson et al (7), individually potted six-week-old tomato plants, kept under optimal conditions in the greenhouse, were employed for the *in vivo* tests. Plants were segregated into isolated groups as follows and inoculated with 48-hour, actively growing cultures of the several strains under study:

1. One group with "M" phase *Phytomonas tumefaciens.*
2. One group with "S" phase *Phytomonas tumefaciens.*
3. One group with "M" phase *Alcaligenes radiobacter.*
4. One group with "S" phase *Alcaligenes radiobacter.*
5. One group with an "S" phase *Phytomonas tumefaciens* culture converted *in vitro* to "M" phase *Alcaligenes radiobacter* (Conversion described later in this paper).

Plant inoculations were made by taking up a little of the culture on a sterile straight needle and puncturing the plant at the internodes (12), averaging three inoculations on one tomato plant. Three plants were used in each group for each isolate under investigation. Sterile needle punctures were likewise made on control plants. Segregation of these various groups was carefully observed and plants were watered by placing water in the bottom of the troughs in which they stood. They were observed daily for any change or appearance of tumorous growth.

Plant Isolations. After six weeks, isolations were made by removing a part of the excess growth adjacent to the plant stem with a sterile razor blade and disinfecting the surface of this plant material by passing it through 50 per cent alcohol and rinsing in sterile distilled water. After this plant material was made homogenous by crushing with a sterile pestle, a drop of this suspension was transferred to yeast-mannitol-mineral salts broth and allowed to grow for 24 hours before streaking on yeast-mannitol-mineral salts agar plates. In the case of crown-gall forming isolates, aniline blue was added to the standard medium to facilitate separation (11). After 48 hours, isolated and typical mucoid colonies, checked first by the Gram stain, were transferred to yeast-mannitol-mineral salt agar slants. Subculturing was carried out by repeating the above procedure until pure cultures were obtained. When the purity of each isolation was certified, antigenic suspensions were prepared and agglutination tests were made with all the antisera on hand.

RESULTS

In Vitro Experiments. The *in vitro* experiments to convert *Phytomonas tumefaciens* "S" phase cells to *Alcaligenes radiobacter* "M" phase cells were successful under two conditions. The first culture was obtained when one drop of a 10^6 dilution of the "S" phase culture was grown in 1 ml. of yeast-mannitol (containing half the amount of mannitol)-mineral salts broth together with 0.5 ml. of heat-killed "M" vaccine of the heterologous organism with 0.2 ml. of the homologous "S" phase antiserum. Succeeding cultures were obtained more rapidly when the strains were grown in mannitol-free, yeast-mineral salts broth and 1.0 ml. of heterologous "M" phase heat-killed vaccine plus 0.1 ml. of the homologous "S" antiserum. It may be possible that the absence of mannitol induced the organism to utilize the carbohydrate in the vaccine material. "M" phase colonies which appeared mucoid were obtained from 48-hour-

old broth cultures on yeast-mannitol-mineral salts agar plates. Gram stains attested the purity of these new isolations and transfers to agar slants of the same medium were made to obtain cultures to check by agglutination tests, the results of which are shown in table 1.

Agglutination occurred only with the *Alcaligenes radiobacter* "M" phase antisera which would seem to indicate the conversion of the *Phytomonas tumefaciens* "S" phase strains to a serological type related to the "M" phase *Alcaligenes radiobacter* strains.

Similar techniques in endeavors to place the *Phytomonas tumefaciens* capsule on the *Alcaligenes radiobacter* "S" phase strains were not successful. When grown in *Phytomonas tumefaciens* "M" phase heat-killed vaccine and *Alcaligenes radiobacter* "S" antiserum, it was found to revert

TABLE 1.—*Results of the agglutination tests of "S" phase Phytomonas tumefaciens strains converted in vitro to Alcaligenes radiobacter "M" phase serological type*

Antisera	Agglutination[a] in dilutions of "S" phase *Phytomonas tumefaciens* antigens									
	40	80	160	320	640	1280	2560	5120	10240	Control
Alcaligenes radiobacter "M"	2+	2+	2+	2+	3+	2+	2+	2+	+	−
Alcaligenes radiobacter "S"	±	±	±	±	−	−	−	−	−	−
Phytomonas tumefaciens "M"	−	−	−	−	−	−	−	−	−	−
Phytomonas tumefaciens "S"	±	±	±	±	−	−	−	−	−	−

[a] Agglutination: − = none, ± = doubtful, 1 = slight; 2 = fair; 3 = good.

to its homologous "M" phase. When grown in *Phytomonas tumefaciens* capsular material in the presence of both the *Alcaligenes radiobacter* "M" and "S" phase antisera, it retained the homologous "S" phase. These results were obtained consistently and indicated by agglutination tests.

In Vivo Experiments. The results of the *in vivo* experiments are in table 2. *Phytomonas tumefaciens* strains in the "M" phase induced a very typical growth in 15 days. A 24-hour-old *Phytomonas tumefaciens* "S" phase culture which showed serological relationship to *Alcaligenes radiobacter* "M" phase culture (Table 1) produced small and abortive tumors, apparent 17 days after inoculation. These tumors became dry and amorphous and ceased developing in a few days with no effect on the growth of the plants. Plant inoculations made after this culture had been carried on laboratory media for 18 days, after the first culture had been obtained, failed to produce tumors. Following inoculations with "S" phase cells of *Phytomonas tumefaciens* tumors formed slowly and were not vigorous.

Plant inoculation with *Alcaligenes radiobacter* "M" phase cells did not result in any tumor formation. "S" phase cells, however, when rubbed

into an aseptically bruised part of the tomato plant stem produced the first evidence of abnormal plant cell growth 3 and 7 days after inoculation. In repeated experiments with all the strains of *Alcaligenes radiobacter* "S" phase cells, a good tumorous growth occurred and resulted in a marked stunting of plant growth. The same was observed with plants inoculated with "M" phase cells of *Phytomonas tumefaciens* strains.

Plant Isolation Results. To determine the validity of tumor production, isolations from the experimental plant tumors were made so that antigenic suspensions could be prepared to use for serological identification

TABLE 2—*Results obtained in 1944 of plant inoculation studies in tomato plants*

Date of inoculation	Inocula	Tumor growth and date of first appearance	Date for well formed tumors	Date of isolation
March 27	*Alcaligenes radiobacter* "M" cells	none		
March 27	*Phytomonas tumefaciens* "S" cells with A. radiobacter capsule[a]	April 13	April 20	May 11
March 27	*P. tumefaciens* "M" cells	April 11	April 13	May 11
April 11	*P. tumefaciens* "S" cells with A. radiobacter capsule[b]	none		
April 11	*A. radiobacter* "S" cells[c]	April 14	April 20	May 11
April 13	*P. tumefaciens* "S" cells	May 10	May 16	May 27

[a] First culture obtained in the laboratory which showed serological relationship to the *Alcaligenes radiobacter* "M" phase strains.
[b] Later cultures which had been carried in the laboratory for sufficient length of time to permit stabilization of the new "M" phase.
[c] Another inoculum which produced tumors within 7 days is not indicated.

(Table 3). Plant passage did not modify the serological properties of *Phytomonas tumefaciens* in the "M" phase, because agglutination occurred only with its homologous "M" phase antisera. In the "S" phase, it was agglutinated only by its homologous "S" and heterologous "S" phase antisera, thereby indicating antigenic similarity to *Alcaligenes radiobacter* "S" phase cells and substantiating the studies reported previously (1). The culture obtained from the tumors produced by inoculating *Alcaligenes radiobacter* "S" phase cells into an aseptically bruised plant internode showed no agglutination by its homologous "S" or "M" phase antisera. On the other hand, this isolate was agglutinated only by the *Phytomonas tumefaciens* "M" phase antisera. This would seem to indi-

TABLE 3.—Serological Tests of Isolates from Experimental Tumors

Antisera	Inocula and plant isolates	Agglutinations at dilutions of								
		40	160	320	640	1280	2560	5120	10240	Control
Phytomonas tumefaciens "M"	*Phytomonas tumefaciens* "M"	3	3	3	3	4	4	4	2a	—
Do	*Alcaligenes radiobacter* "S"	2	2	2	2	2	2	2b	—	—
Do	*Phytomonas tumefaciens* "S"	1	1	1	—	—	—	—	—	—
Do "S"	Do	2	3	3	3	3	3	2	—	—
Alcaligenes radiobacter "M"	*Phytomonas tumefaciens* "M"	—	—	—	—	—	—	—	—	—
Do	Do "S"	—	—	—	—	—	—	—	—	—
Do "S"	*Alcaligenes radiobacter* "S"	1	1	1	1	1	1	1	—	1
Do	*Phytomonas tumefaciens* "S"	1	1	1	1	1	1	1	—	1

a Agglutination: — = none; 1 = slight; 2 = fair; 3 = good; 4 = excellent.   Agglutinated by heterologous "M" antisera and not by homologous
"M" or "S" phase antisera.

b Shows serological relationship to *Phytomonas tumefaciens* "M."

cate the *in vivo* conversion of *Alcaligenes radiobacter* "S" phase to the *Phytomonas tumefaciens* "M" phase.

In the case of *Alcaligenes radiobacter* "M" phase inoculum and the *in vitro* converted *Phytomonas tumefaciens* strains, no contributable material for serological tests was obtained since no tumors were produced.

DISCUSSION OF RESULTS

The results of this study seem to indicate that the organisms recognized as *Alcaligenes radiobacter* and *Phytomonas tumefaciens* comprise a single species and in their usual "M" phases bear the same relation to each other as do the types of the pneumococcus (2, 6) to one another. The failure, under conditions of the experiments, in placing the *Phytomonas tumefaciens* capsule on the *Alcaligenes radiobacter* "S" phase cell, but the success of the *in vitro* attempts with the "S" phase of *Phytomonas tumefaciens* suggests that the latter represents a relatively unstable form. It would appear that the "M" phase recognized as *Alcaligenes radiobacter* is the normal and stable phase in nature and that the "M" phase recognized as *Phytomonas tumefaciens* represents a transitory adaptation. Differences in the relative stability of the strains of these organisms were significant throughout these investigations. *Alcaligenes radiobacter* stimulated better antibody production than did *Phytomonas tumefaciens* (1). In decapsulation studies, *Phytomonas* strains proved less resistant to the presence of the homologous antisera and lost the capsular substance in the course of 7 serial transfers (1). *Alcaligenes* strains, on the other hand, were resistant to homologous antiserum of higher titer and as many as 19 transfers were required to remove the capsule (1). Moreover, in capsule conversion attempts, the "S" phase of *Phytomonas* strains slowly yielded and acquired the capsule of *Alcaligenes radiobacter*, while in the *in vitro* attempts to convert the "S" phase of *Alcaligenes radiobacter* to the "M" phase of *Phytomonas tumefaciens* that was not accomplished, but conversion was only brought about *in vivo*.

It is interesting to note that Hendrickson et al (7) suggested that in view of the results obtained in their study it is possible to bring about a transference of *Phytomonas tumefaciens* into an organism possessing the important characters of the "so-called" *Alcaligenes radiobacter*. The results of our studies would seem to indicate that this was accomplished and may offer an explanation for the baffling similarities which have persistently resisted the efforts of notable workers in this field (7, 9, 10, 11) in attempts to differentiate *Phytomonas tumefaciens* and *Alcaligenes radiobacter* by morphological and physiological characteristics.

SUMMARY

For the strains under investigation in this study, conversion *in vitro* of *Phytomonas tumefaciens* to *Alcaligenes radiobacter* was brought about by

placing the *Alcaligenes radiobacter* capsule on the *Phytomonas tumefaciens* "S" phase cell.

Although difficulty was encountered in attempts to effect the conversion of *Alcaligenes radiobacter* to *Phytomonas tumefaciens in vitro,* this conversion was effected *in vivo* by inoculating suitable host plants with the *Alcaligenes radiobacter* strains in the "S" phase.

The conversion of *Phytomonas tumefaciens* to a form serologically identical with *Alcaligenes radiobacter* in the "M" phase was accompanied by loss of the ability to attack a susceptible host plant.

The removal of the *Alcaligenes radiobacter* "M" capsule changed this organism from a state of avirulence to one of significant virulence which in the presence of a suitable host induced the formation of a capsule of the *Phytomonas tumefaciens* serological type.

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PAPAYA VIRUS DISEASES WITH SPECIAL REFERENCE TO PAPAYA RINGSPOT[1]

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INTRODUCTION

Papaya (*Carica papaya* L.) is the third most important fruit crop grown in the Territory of Hawaii, being exceeded in commercial value only by pineapple and banana. In 1945 a previously undescribed papaya disease was encountered at Kailua on the island of Oahu. The discovery of the disease, named papaya ringspot, was published by Lindner *et al.* (32) in 1945. Experiments reported in abstract form by Jensen (24, 25) in 1946 and 1947 demonstrated the virus nature of the disease, its incubation period in the plant, and that *Myzus persicae* (Sulzer) and three unnamed *Aphis* species were capable of transmitting the virus. Failure to infect papaya with five common virus diseases occurring on Oahu or to transmit ringspot virus to plants other than papaya was also reported.

In 1947 Hendrix and Matsuura (19) reported juice transmission of ringspot virus to healthy papaya by the use of carborundum and also by hypodermic injection. The disease was not found to be transmitted through the seed.

Lindner *et al.* (33) in 1947 reported field observations on the rate of disease spread and on the effect of adverse environmental conditions on ringspot trees. Holmes *et al.* (22) in 1948 published results of a ringspot disease survey made on the main islands of the Hawaiian group. The disease was found only on Oahu. The report also stated that 15 varieties, strains, and hybrids of papaya tested were susceptible to the disease. Possible control procedures were also discussed.

The present paper reports the results of experiments on papaya ringspot disease carried out on the island of Oahu during 1945 and 1946. Included is a discussion of the symptoms and incubation period of the disease in the foliage, stem, and fruit of the papaya, rate of virus movement through the plant, and host range investigations. The virus-vector relationships of papaya ringspot are discussed in a separate paper (26). The writer's departure from Hawaii, terminating the investigation, accounts for the preliminary nature of certain phases of the study, such as virus movement through the plant. Since most of the known facts regarding the occurrence and types of papaya virus diseases throughout the world have been reported only within the past 2 decades and because this information has never been summarized, a review of the reported viruses of

papaya is included. The possible relationships of some of these viruses are also discussed.

<div style="text-align:center">LITERATURE REVIEW</div>

The first report of a papaya disease being attributed to a virus was made by Smith (47) in 1929 in Jamaica. In later accounts (47) he stated that papaya mosaic was primarily responsible for the absence of large-scale papaya cultivation in Jamaica. Smith described the disease as causing yellowing and mottling of the leaves, accompanied by reduction of the terminal leaves to a pencil point. The larger leaves meanwhile hang down and the plant gradually dies. Buds are often put out lower down on the stem but they usually also become diseased and die. Later reports on the Jamaica virus were published by Edwards (16) in 1933, by Larter (30, 31) from 1935 to 1943, and by Martyn (35) in 1942. No transmission experiments have been reported from Jamaica although Larter tested 29 types of papaya for resistance to the disease in the field. In 1937 one of these, *Carica cauliflora* Jacquin from Panama, was reported to be immune to the disease.

A curly-leaf disease of papaya was reported from Santo Domingo in 1930 by Ciferri (13). Symptoms include curling and twisting of the younger leaves to form cushions, yellow and green mosaic spots on the older leaves, followed by yellowing and drying up of the leaves, both old and young, within two months. A succession of new growth develops which in turn becomes diseased and falls from the plant. These conditions last for several months to one or two years before the plant dies. The upper part of the main stem of diseased trees develops irregular, elongate suberous epidermal growths resulting from the formation of layers of cork under the epidermis.

Although Ciferri reported transmission of curly leaf disease to 29 of 78 healthy plants by inoculation with latex from infected plants, he was uncertain as to the cause of the disease and did not suggest a virus origin. His positive cases varied from one out of 18 in one experiment to 18 out of 20 in another. Since Ciferri did not report that his plants were protected from natural spread and no controls were mentioned, latex transmission of curly-leaf disease needs confirmation.

Three virus diseases, bunchy top, die-back, and mosaic, occur in Puerto Rico. Bunchy top, first reported by Cook (14) in 1931, has been the major limiting factor in papaya production on the island since 1937 (7). J. H. Jensen (27, 28) in 1938 and 1939 described the disease symptoms and transmission experiments. Infected plants become stunted, produce chlorotic leaves with rigid, short petioles which extend horizontally rather than nearly vertically from the main stem. In advanced stages the trees are denuded except for a few small leaves at the top. The petioles and stem develop dark green, elongate streaks. Diseased plants produce very little latex from wounds. A high percentage of diseased trees is killed.

Attempts to transmit the virus mechanically and by grafting were unsuccessful. J. H. Jensen found a new species of leafhopper breeding on papaya which was described by Oman (38) in 1937 as *Empoasca papayae.* Nymphs and adults of this insect were caged on young, healthy papaya plants for 10 days.   Three months after the insects were removed, the test plants developed symptoms resembling those of bunchy top. Soon thereafter the plants died as a result of root-rot fungus.   In the meantime Jensen left Puerto Rico and the experiment was not repeated until several years later.   In 1946 and 1947 Adsuar and Sein (3, 43) reported experiments which confirm the identity of *Empoasca papayae* as the vector of papaya bunchy top virus.

Kevorkian (29) in 1938 reported that the lace bug, *Corythuca gossypii* L., and the stink bug, *Nezara viridula* L., failed to transmit bunchy top. Of particular interest is the observation that bunchy top plants appear to be more acceptable as host plants of the lace bug than are healthy plants. Colonies are usually found only on diseased plants, and the insect is present on most diseased plants.   The disease usually precedes the infestation.

Papaya die-back, mentioned briefly by Adsuar (4) in 1946, is characterized by severe chlorosis, defoliation, and necrosis of the apex of the stem.   The symptoms resemble those of bunchy top but are much more severe.

In 1946 Adsuar (4, 5, 6) described a papaya mosaic from Puerto Rico which is transmissible by mechanical inoculation, by grafting, and by means of the green citrus aphid, *Aphis spiraecola* Patch.   Plants develop symptoms in from 8 to 21 days.   Progressive symptoms include chlorosis, mottling, wrinkling, and puckering of the top leaves.   As the disease progresses, the plant becomes stunted and the leaves are severely deformed and may be reduced to filiform structures.   The stem develops dark green, elongate streaks and the fruits have green and dark brown rings.

In 1945 Goenaga (17) reported that a leaf-roll virus of tobacco had spread in Puerto Rico to nearby plants representing several plant families. Young papaya trees reportedly acquired the disease when placed in contact with diseased potato plants.   The symptoms on papaya resemble those of bunchy top.   Since Goenaga's observations with regard to papaya have not been confirmed experimentally, the identity of the papaya disease and the conclusion that it was a tobacco virus and that it had spread from potato must yet be proved.

Stell (48) in 1937 stated that no commercial cultivation of papaya in Trinidad had been attempted and that such an undertaking would probably fail.   Papaya mosaic was listed as the first of two major diseases of this plant which would hinder successful cultivation.   Baker (9) in 1939 described Trinidad mosaic symptoms and stated the disease was severe in all districts of the colony.   Marked mosaic of the leaf lamina and watersoaked areas or oil spots on the petioles constitute the first symptoms of the disease.   According to Baker's illustration, the leaf mosaic consists of

conspicuous chlorotic spots and irregular chlorotic areas spreading out from the veins and veinlets. No new terminal leaves are produced after the growing point becomes diseased. In about two months after symptoms appear the top of the tree dies and secondary rotting sets in which is arrested one or two feet below the apex. The tree may be killed outright in a short time. However, usually axillary shoots are produced, the lower of which sometimes remain healthy and bear fruit.

Trinidad mosaic has not been transmitted experimentally. Baker tested 14 species of homopterous insects with negative results. On the basis of symptoms and spread there can be little doubt that the disease is due to a virus despite Castelli's (12) contention in 1939 that the disorder is a result of improper soil conditions.

Acuna and Zayas (1, 2) in 1940 and 1946 described two diseases of papaya in Cuba apparently caused by viruses. One, called type A, is considered to be the same as bunchy top in Puerto Rico. Type B, known as Cotorro mosaic and limited to Havana, is more destructive than type A. The leaves develop chlorotic, translucent areas which extend out from the veins. New leaves become twisted and otherwise distorted. The interveinal tissue is puckered and reduced to the extent that the veins are crowded together and often run parallel. The bases of the young petioles and sometimes the stems develop green or greenish-red areas which are annular in form. As the plant grows, the color of the spot becomes normal in one zone, thus changing the form from a ring to a hook or a U with unequal arms. Spots sometimes also appear on the fruit. In some plants the basal leaves persist and the upper part of the stem is completely denuded or carries a new flush of leaves at the apex with the intervening space bare. In contrast to bunchy top, the latex flows freely from plants having Cotorro mosaic.

In South America, papaya diseases attributed to viruses have been reported from British Guiana, Brazil, and Venezuela.

In 1934 Martyn (34) reported a papaya disease from British Guiana, described only as curly leaf, which spread among the seedlings in a nursery.

Bitancourt (10) in 1935 published a short note describing a mosaic of papaya observed on plantations in São Paulo, Brazil. It is characterized by vein chlorosis of the older leaves and in the young leaves by malformation, stunting, reduction of the leaf blade, and by leafroll. Goncalves-Silva (18) in 1941 also reported mosaic as one of the diseases of papaya in Brazil and stated that it may sometimes be confused with the effects produced by mites on papaya.

An undescribed papaya mosaic was reported by Mueller (37) in 1941 to be extremely severe in Venezuela except in the Andes region.

An undescribed mosaic disease of papaya was reported for the first time from Rhodesia in 1940 by Hopkins (23).

In India at least two distinct papaya viruses have been reported.   In 1939 Thomas and Krishnaswami (51) described a leaf-crinkle disease found at Coimbatore in South India.   Instead of growing erect, the stem acquires a zigzag appearance.   The leaves are crinkled and the margins curve downward and inward simulating an inverted cup.   Plants which become diseased while small grow without appreciable stunting and retain symptoms throughout life.   The disease was transmitted by grafting, symptoms developing in 120 days.

In 1940 a disease called papaya leaf curl was reported from the Madras Presidency (8).   It was transmitted to healthy plants by grafting.

In 1946 Sen *et al.* (44) described a leaf-curl disease of papaya from Bihar as follows: ''A serious disease of the papaya initiating with the symptoms of etiolation and curling of leaves, in advanced stages showing crippled leaves and fruits of various degrees and ultimately bringing about premature death of the plant has been observed.''   The disease was not seed-borne, but was juice-transmissible.   The report asserts further that the disease could be initiated independent of inoculation by waterlogging the soil in which the papayas grow.   However, symptoms resulting from growth in waterlogged soil were undoubtedly not due to a virus.

Su (50) in 1934 reported a papaya disease in Burma, considered to be due to a virus, which causes crinkling and distortion of the leaves and in severe cases adversely affects the fruits.

A virus disease of papaya was reported to occur in Kwangtung Province, China, by Tu (52) in 1932 and by Ho and Li (20) in 1936.   Affected leaves are curled and stunted, and bear chlorotic patches between the veins.   The margin of the inter-veinal lamina is often deeply indented.   Frequently the trees are defoliated except for a few leaves at the top.

Yellow crinkle of papaya was reported from Queensland, Australia, by Morwood (36) in 1931, Simmonds (45, 46) in 1934, 1936, and 1937, and by Da Costa (15) in 1944.   This disease causes the older leaves to turn yellow and droop.   Between the veins of young leaves thin, translucent areas develop and later drop out.   As these leaves mature they remain light yellowish green and appear crinkled.   The tips of the segments of very young leaves curl downward and inward.   In advanced stages the tree is bare of leaves except for a few stunted, distorted leaves at the apex.   Yellow crinkle has not been transmitted mechanically or by insects.

A die-back disease of papaya was described from Java in 1931 by Rant (42), who reported bacteria as the causal agents.   The symptoms resemble somewhat those caused by virus in some regions of the world, and Brierley (11) listed it as a virus disease in 1945.

Parris (39, 40, 41) from 1938 to 1941 discussed a new papaya virus disease discovered at Waialua and Lualualei, Oahu, in 1937.   Mechanical transmission was obtained in over 75 per cent of the plants inoculated.   Symptoms appeared in from 16 to 21 days after inoculation.   Diseased plants are stunted and the leaves become chlorotic.   The petioles of dis-

eased leaves bend down from their point of attachment on the stem. Hydrotic streaks occur on the petioles of diseased leaves and on the main stem. These streaks are linear, dark green, and slightly raised. Diseased leaves fall in four to six weeks after first symptoms, the stem is left bare except for a few stunted, distorted leaves at the apex and frequently a fringe of lower leaves which developed prior to symptom expression. Fruits on diseased trees "bleed" profusely. The incidence of this disease gradually diminished in the field and it has been observed only occasionally since 1939. Holdaway and Look (21) in 1940 reported negative results from preliminary vector experiments involving 8 species of insects.

Papaya ringspot disease, discussed in the succeeding pages of this paper, is the second papaya virus disease to be described from Hawaii. The restricted distribution of the disease on Oahu and its rapidity of spread within infected plantings, coupled with its deleterious effect upon commercial acreages at Kailua, suggest that it has been established in papaya in Hawaii for a relatively short time. The origin of the disease and its possible occurrence on islands or continental areas outside of Hawaii are not known.

MATERIALS AND METHODS

All papaya plants used in this investigation were of the Solo variety.

Experimental plants varied in age from 4 weeks to 15 months and in height from 2 inches to 15 feet at the time of inoculation. Test plants were grown in the greenhouse in cans varying in size from one pint to one gallon. Papayas placed in the field were usually from 6 to 20 inches tall at the time they were transplanted. In experimental field plots the plants were spaced either 2½ or 3 feet apart in the row with 3 feet between rows. In commercial plantings the trees are usually spaced 10 to 12 feet apart.

At least one check tree was maintained for every tree used in a transmission test. In the greenhouse no control plants acquired the disease. In the field, natural spread to control plants occurred in all plots containing diseased trees if the plots were maintained for several months. Symptoms on the first control plants to become infected by natural spread usually appeared in 4 to 6 weeks after the first series of experimentally infected trees in the plot developed symptoms. When plots began to show evidence of natural spread of the disease they were not used further in transmission experiments.

PRIMARY FOLIAGE SYMPTOMS

The symptoms of papaya ringspot disease occur in the foliage, fruit, and main stem of the plant. Considerable variation exists in the degree of symptom expression.

The first evidence of the disease in plants inoculated with ringspot virus by means of *Myzus persicae* is a puckering or bulging of the leaf tissue between the secondary veins and veinlets on the upper surface of the young terminal leaves (Fig. 1). A tendency to roll downward and inward is

also apparent on the margins and distal points of the leaves. These symptoms, which usually appear in two weeks, are particularly pronounced in young plants growing rapidly in the field. As the affected leaves become larger, the puckering becomes less conspicuous and the leaves acquire a mildly rugose appearance. This is due to small, scattered convexities and concavities formed by the leaf surface between the smaller veins Frequently most of these blister-like bulges are produced on the lower surface of the leaves, leaving portions of the upper surface pitted. This is especially true on plants which have had the disease for several months.

As they approach maximum expansion, affected leaves on field trees show a distinct tendency to roll upward along the margins. This is most

FIG. 1. A. Primary symptoms of papaya ringspot in young leaf of field tree, showing puckering of tissue between veinlets and downward rolling of margins and tips of leaflets. B. A healthy leaf of approximately the same age.

conspicuous on trees which have been diseased for several months. This characteristic is in contrast to that of young diseased leaves to roll downward and inward along the margins.

The general color of affected leaves is lighter green than normal. Moreover, the green color of the interveinal tissue is not of uniform quality but is characterized by irregular areas which are lighter green than the rest of the leaf. These lighter areas occur primarily along the smaller veins and veinlets but frequently extend throughout the interveinal tissue.

FACTORS AFFECTING PERIOD OF DEVELOPMENT OF PRIMARY SYMPTOMS

The time necessary for ringspot virus to produce primary foliage symptoms in papaya varies from 9 to 39 days after inoculation. An incubation period exceeding 21 days is rare, however.

The most important factor determining the length of the incubation period is the growing condition of the plant. However, the site of inoculation also has a definite though less marked effect. Plants making rapid, vigorous growth typically express symptoms in from 9 to 14 days when inoculated by means of aphids. Plants whose growth, at the time of inoculation, has been arrested or severely retarded by growing too long in small cans usually require a longer time for symptom production. In one experiment involving 8 young plants making rapid growth in gallon cans in the greenhouse, the incubation period varied from 9 to 15 days with an average of 10.5 days. In another experiment 31 plants, 8 to 10 inches tall and making slower growth in quart cans, developed symptoms in from 11 to 21 days with an average of 15 days.

An experiment was conducted to determine the effect of inoculation site on incubation period of the disease in field papaya trees 3 to 4 feet in height. Forty-four trees were infected by means of *Myzus persicae* caged on leaves of varying maturity.

Plants inoculated at the growing point and in the youngest three expanding leaves produced symptoms in from 10 to 15 days with an average of 11.1 days. Plants inoculated in the 4th to 11th youngest leaves developed symptoms in from 12 to 20 days with an average of 14.6 days. There was no difference in incubation period between plants inoculated in the 4th youngest leaf and those inoculated in the 10th or 11th youngest leaves. Sixty noninoculated check plants scattered throughout the plot remained disease-free.

MOSAIC OR SECONDARY FOLIAGE SYMPTOMS

Marked mosaic or mottle symptoms develop in the leaves of the tree canopy and in the small axillary leaves (Fig. 2). The development of this symptom requires a longer period of time than does the initial foliage symptom and is favored by the cool, cloudy weather common during the winter months in Hawaii. The leaf mottle results largely from accentuation of the differences in color intensity which are present but inconspicuous during the earlier stages of the disease. Reduced light seems to be a requirement for the production of prominent mottle symptoms.

During the winter months the mottle in the leaves is very marked; in contrast, during summer this character becomes poorly defined. However, diseased trees which were grown to a height of 9 and 10 feet under crowded field conditions ($2\frac{1}{2}$–3 feet apart) developed a striking mottle pattern in the shaded axillary leaves during the summer months even though the leaves of the canopy showed little or no mottle. Diseased plants, spaced far enough apart to permit more normal exposure of the axillary leaves to sunlight, developed only mild mottle in these leaves during the summer months.

The time necessary for the production of mottle symptoms is variable, depending to a large extent on the prevailing climatic conditions. The

shortest period recorded for the development of a strong mottle in the top foliage was 24 days after inoculation. The more typical time period during the winter months, however, is six weeks after inoculation. In contrast to this, plants inoculated during early summer did not develop even mild top mottle until three months after inoculation although the shaded axillary leaves on some of these plants had a well-defined mottle in 40 days.

FIG. 2. A. Axillary leaf from a ringspot papaya tree, showing mosaic symptoms and leaf rugosity. The axillary leaves were shaded by the foliage canopy of trees growing close together. Mottle symptoms were produced in these leaves during the summer in the absence of well-defined mottle in the top leaves of the tree. B. Healthy axillary leaf, grown in shade, from a non infected tree growing in the same plot as "A".

STEM SYMPTOMS

The main stem of papaya plants growing in small cans at the time of infection frequently develops dark green spots and streaks of an oily or water-soaked appearance. These are usually most common on the middle two-thirds of the stem. This symptom is characterized first by the development of a number of distinct, round spots approximately $\frac{1}{16}$ inch in diameter. These range in number from 4 to over 100 on the stem of a single plant 10 inches in height. In severe cases the spots increase rapidly in number and definition during the first few days after they become discernible. As they increase in number the water-soaked stem spots coalesce to form larger areas which frequently appear as elongate streaks.

The time required for the development of the dark green spots and streaks on the stem varies, depending apparently on the growing condition of the plant. In one experiment with 39 test plants (31 growing in quart cans and 8 in one-gallon cans), the shortest time recorded for the production of stem symptoms on plants infected by means of *Myzus persicae* was

13 days. The longest incubation period was 29 days. The average for the entire group was 17.7 days. Typically, the stem symptoms develop within a few days after the primary foliage symptoms have appeared. In several instances the foliage and stem symptoms appeared the same day. In 2 cases stem symptoms were recorded for plants which failed to develop recognizable foliage symptoms. Nine of the 39 trees produced foliage symptoms but failed to develop stem symptoms.

The stem symptom has not been observed on field trees which were well established and growing rapidly at the time of infection. It has, however, appeared on plants which were transplanted to the field from small cans within a few days after their inoculation by means of aphids. In these cases the plants made little growth during the first 10 days in the field. The water-soaked streaks persisted for several weeks.

Observations indicate that the stem-spotting symptom is correlated with severe retardation of growth in diseased plants. Papayas grown in small cans grow much slower than do plants in the field. It is typically under these conditions of slow growth that stem symptoms appear.

As a rule the plants which were making such poor growth in small cans that the foliage symptoms were indistinct or mild developed severe water-soaked spotting over the stems. Conversely, plants which produced the strongest foliage symptoms typically developed fewer and less conspicuous spots on the stems.

FRUIT SYMPTOMS

Papaya fruits show virus symptoms, as rings or spots, both during their early stages of development and as they approach maturity. The flavor and texture of infected fruits, however, are not impaired by the disease.

Young fruits, one to 3 inches long, frequently develop small, light green rings on their surface. The rings on young fruits are usually relatively few in number and inconspicuous. They become even less distinct as the fruits become larger and darker green. Young fruits bearing ring symptoms were marked and the position of the rings on the individual fruits was recorded. When these fruits became mature green and developed typical yellow rings, the position of the light green rings on the young fruits had no direct relation to the position of the yellow rings on the mature fruits. The rings on small young fruits were usually localized as one or 2 compact groups of 6 to 12 rings per group. The yellow rings produced on these fruits at maturity were numerous and were scattered over most of the fruit surface. Many of the fruits on experimentally infected trees developed no detectable rings or spots while the fruits were small. However, yellow rings invariably appeared on all of the fruits as they approached maturity.

The yellow spots and yellow rings with green centers (Fig. 3, B) on mature green fruits provide the most striking and reliable symptom of the disease. The size of the spots ranges from approximately $\frac{1}{16}$ to $\frac{1}{8}$ inch in

diameter. The rings vary in size from ⅛ to ¾ inch in diameter. The number of spots or rings on a single fruit may vary from a few to over 150.

FACTORS DETERMINING TYPE OF FRUIT SYMPTOM

The developmental stage of a given fruit at the time the plant becomes infected with ringspot virus determines the type of symptom produced on that fruit.

Typically, fruits ⅞-grown or older at the time of infection ripen without evidence of the virus. Fruits ⅝-mature or somewhat younger at the time of infection develop yellow spots at the mature green stage. These appear in from 6 to 8 weeks after the tree becomes infected. Fruits maturing

FIG. 3. A. Papaya leaf from a tree naturally infected with ringspot in a commercial field at Kailua, Oahu. Experimentally infected trees, receiving good care, have failed to develop such severe symptoms. B. Papaya fruit showing yellow rings with green centers caused by ringspot virus. This symptom appears a few days before the maturing fruit changes color from green to ripe yellow.

during the first 3 weeks after the first spotted fruit appears likewise exhibit yellow spots as the only disease symptom. During the next 3 to 5 weeks, maturing fruits develop both solid yellow spots of enlarging size and small rings, i.e., yellow spots with very small green centers. With increasing time the rings produced on newly ripening fruits become progressively larger and more numerous while the number of solid yellow spots decreases. Three months after the first yellow spots have appeared the symptom on maturing fruits has changed to a predominance of rings with only a few spots remaining. If no fruits have been formed, or the oldest fruits are not more than approximately ½ grown at the time the plant acquires the disease, the first fruits to mature, as well as all mature fruits subsequently produced, carry well-defined rings and few if any spots.

Table 1 indicates the relationship of fruit development at the time of infection to the type of symptoms on maturing fruits. The time periods vary considerably from tree to tree and from season to season depending upon the growth rate. Two trees, infected during the winter and producing ripe fruit at the time of infection, required 47 and 61 days, respectively, after infection to produce the first spotted fruit and 71 and 82 days, respectively, to produce the first fruit with rings.

TABLE 1.—*Effect of stage of fruit development at time of infection on symptoms on maturing fruits*

Approximate stage of fruit when plant was infected.[a]	Symptoms on maturing fruits	Approximate time from infection to fruit maturity[a] (in days)
⅚ grown	No symptoms	25
⅔ grown	Small, solid yellow spots only. No rings.	50
½ grown	Predominantly solid yellow spots; a few small, distinct rings.	75
⅓ grown	Rings common and enlarging; solid yellow spots still in majority.	100
⅙ grown	Predominantly well defined rings; only a few solid yellow spots.	125
Newly fertilized flowers	Rings only	150

[a] The figures listed fluctuate from tree to tree and vary with the season. Dr. Wm. B. Storey, Horticulturist at the University of Hawaii, has informed the writer that in Manoa Valley, where these tests were conducted, the average time for papaya fruits to reach the firm ripe stage of maturity after pollination is 150 days. Time is shorter than 150 days for fruits starting development during March and April and longer for those starting during November and December.

EFFECT OF PAPAYA RINGSPOT VIRUS ON PLANT GROWTH AND VIGOR

In addition to the rugosity and mosaic mottle produced in papaya leaves, ringspot virus also causes a reduction in size of the leaf lamina and in length of the petiole. The tree as a whole is also stunted by the disease. The degree of stunting, however, varies considerably. In the experimental plots of the University of Hawaii, at Honolulu (on the lee side of the island of Oahu), where the plants were fertilized and irrigated regularly, papayas, experimentally infected when 10 inches high, grew moderately well and produced fruit despite the presence of the disease. These plants were not, however, as large and vigorous as were the non-diseased trees. In the commercial papaya plantings in the vicinity of Kailua, on the windward side of Oahu, however, effects of the disease are much more severe and infected trees usually become commercially unprofitable within a year or two after becoming infected. In these plantings adverse environmental conditions, such as seasonal drought, affect ringspot trees more severely

than non-infected trees. Stunting of all parts of the tree becomes extreme and the loss of chlorophyll in diseased foliage frequently leaves much of the interveinal lamina translucent (Fig. 3, A).

Ringspot virus has no effect on the normal flow of latex from wounds made in stem, leaf, or green fruit tissue.

RATE OF VIRUS MOVEMENT THROUGH PAPAYA PLANT

Ringspot virus moves rapidly upward through the branches and main stem of the plant. Symptoms typically appear in the terminal foliage 13 or 14 days after the plants are inoculated on mature leaves. The incubation periods of Hawaiian papaya mosaic or Waialua disease (16–21 days) and of mosaic in Puerto Rico (8–21 days) are also short.

The rate of virus movement downward in papaya plants has received little investigation. Smith (47) in 1932 and Edwards (16) in 1933, reporting on the papaya mosaic in Jamaica, stated that a reasonable proportion of "recoveries" could be obtained if diseased trees were cut back to the soil level as soon as the first symptoms of the disease appeared. Furthermore, in some cases the lower branches remained healthy if the diseased main stem, dying with the disease, were removed.

The Waialua disease of papaya in Hawaii, described by Parris (39), sometimes killed the growing point without invading the branches later produced lower down the tree. Moreover, if diseased trees were decapitated, the new growth developing from the basal portion of the stem was healthy or some shoots were healthy and some diseased.

In Trinidad, Baker (9) in 1939 observed that the top of mosaic-diseased trees usually died in 8 or 9 weeks after symptoms appeared. This was followed by secondary rotting which stopped 1 or 2 feet below the original apex of the tree. A large number of axillary buds were subsequently produced below the dead point. The upper buds became diseased at once but frequently the buds lower down the tree grew to large size and produced fruit.

These results suggest that some papaya viruses are slow in moving downward through the main stem of the plant.

The present studies of papaya ringspot disease did not include decapitation of large, recently infected papaya plants to determine how rapidly the virus invaded the lower portions of the main stem. Several plants, infected when one foot or less in height, which had been diseased for 3 or 4 months, were cut off at or near the ground level. The new shoots produced from the base of these plants were all diseased.

A single preliminary experiment was conducted to determine the time required for virus to move from the point of inoculation in one branch of a dichotomously branched papaya to the other branch and there to produce symptoms. Although only 2 plants were available for use in the test, the results justify brief mention.

Two papaya plants 3½ feet tall were cut off at a point 8 inches above the ground on February 28, 1946. Each plant subsequently produced 2 new lateral shoots which arose on opposite sides of the main stem. One branch on each plant originated at a point 3 inches above the point of attachment of the second branch. On April 6, 1946, when the new shoots were each approximately 14 inches long, 100 infective *Myzus persicae* were caged on the terminal of one branch on each tree. ' Symptoms appeared in the new growth of each of the inoculated branches in 12 days. The terminal growth on the noninoculated branch of each of the 2 trees did not develop symptoms until 38 and 44 days, respectively, after the trees were initially inoculated. The original inoculation was made on the upper branch of one plant and on the lower branch of the other.

These results indicate that ringspot virus movement is relatively slow downward through the branch of a rapidly growing papaya plant. In these 2 tests when the virus moved down a branch and into the main stem in order to enter a non-infected shoot on the same plant and there produce symptoms, a time period was required which was approximately 3 times as long as the incubation period in the inoculated branch.

FAILURE TO INFECT OTHER SPECIES OF PLANTS

Extensive host range studies have not been reported for any of the papaya viruses listed in the literature. The botanical affinities of the genus *Carica* are not well known. According to Storey (49) papaya, now placed in the Caricaceae, has at one time or another been placed in the following different families: Passifloraceae, Cucurbitaceae, Bixaceae, and Papayaceae

The native home of the papaya is considered to be Central America, the British West Indies, or Mexico. No closely related plants are known to occur in Hawaii, the passion flower vine, *Passiflora* spp., possibly being the nearest in botanical relationship.

Experiments were conducted in Hawaii to determine whether papaya ringspot virus could be transmitted by means of aphids to hosts other than papaya. Nearly all of the test plants used were grown from seed in the greenhouse and were fed upon by infective aphids while the plants were still small. Three weeks after inoculation, non-infective *Myzus persicae* were placed on the test plants, allowed to feed for 24 hours, and then transferred to healthy papaya seedlings.

Table 2 lists the plants used in the host range investigation. Twelve plant families were tested. These were represented by 16 species of plants, most of which occur commonly in the papaya ringspot disease area. Virus symptoms failed to develop in any of the plants tested and previously non-infective aphids failed to recover ringspot virus from 11 species used in recovery tests.

It is of further interest that of the papaya viruses reported in the literature, none is known to infect other hosts.

VIRUSES TESTED ON PAPAYA

A number of virus diseases occur in cultivated and wild plants in the papaya ringspot area of Oahu. With the exception of the mosaic diseases of pepper, Commelina, and Crotalaria this is the first report in the literature of the diseases discussed below occurring in Hawaii. Experiments were conducted to determine if any of these diseases could be transmitted

TABLE 2.—*Plant species tested as possible hosts of papaya ringspot virus. Inoculations were made by means of infective Myzus persicae. All tests were negative.*

Plant family	Test plant	Number of plants inoculated	No. aphids used per test
Commelinaceae	*Commelina diffusa* Burm. (Wandering Jew)	3	100
Crassulaceae	*Bryophyllum calycinum* Salisb.	2	150
Chenopodiaceae	*Beta vulgaris* L. (Table beet)	49	30
Compositae	*Lactuca sativa* L. (Lettuce)	27	20
Cucurbitaceae	*Cucumis sativus* L. (Cucumber)	20	100
Cruciferae	*Brassica chinensis* L. (Pakchoi)	72	50
Malvaceae	*Malvastrum coromandelianum* (L.) (False mallow)	4	50
Nyctaginaceae	*Mirabilis jalapa* L. (Four o'clock)	12	50
Leguminosae	*Crotalaria incana* L. (Fuzzy rattle pod)	9	100
Passifloraceae	*Passiflora foetida* L. (Passion flower)	2	30
	P. pfordti (Passion flower) (= *alatocaerulea*)	3	100
Portulacaceae	*Portulaca oleracea* L. (Purslane)	20	75
Solanaceae	*Lycopersicon esculentum* Mill. (Tomato)	21	50
	Capsicum frutescens L. (Pepper)	13	50
	Solanum tuberosum L. (Potato)	10	500
	Nicotiana tabacum L. (Turkish tobacco)	21	200
		288	

to papaya by means of aphid vectors, and in particular to determine if any produce symptoms in papaya which resemble those of ringspot disease.

The literature contains no report of papaya having been experimentally infected with any virus occurring in another plant species. None of the viruses tested during this study produced symptoms in papaya and in no instance were the experimental viruses recovered from papaya.

Myzus persicae was used in all tests except those involving Crotalaria mosaic. In the Crotalaria tests, *Aphis gossypii* was used. This species also constituted part of the aphid population in tests with Commelina mosaic and with pepper mosaic.

Hibiscus ringspot.[3] Several hibiscus plants in Honolulu were found

[3] Brought to the attention of the writer by Dr. F. O. Holmes of the Rockefeller Institute for Medical Research.

to carry ringspot symptoms on the leaves. The rings, approximately $\frac{1}{8}$ of an inch in diameter, are lighter green than normal tissue and contain dark green centers. On some plants the rings are very distinct; on others they are scattered throughout a more diffuse mosaic area and are less conspicuous. The hibiscus flowers on some plants on Oahu and Kauai also carry a large diamond-shaped pattern of breaking in the color of the petals. Whether this is caused by the same virus producing ringspots on the leaves has not been determined.

Scaevola frutescens ringspot. This plant is known in Hawaii as beach "naupaka." A virus disease of *S. frutescens* was first noted June 21, 1946, in the Kailua area of Oahu.[4] The symptoms are very variable and include chlorotic, etched ring and oak leaf patterns and mottling and crinkling of the leaves. On some leaves, rings occur in a concentric pattern. This disease was subsequently observed on the island of Kauai where no papaya ringspot is known to occur.

Passiflora foetida mosaic.[3] Conspicuous mosaic symptoms occur in the leaves of this plant in Honolulu.

Passiflora pfordti mosaic. This plant, a hybrid resulting from crossing *P. alata* Dry and *P. caerulea* Linn., carries virus symptoms in Honolulu.[5] The younger leaves bear small yellow spots while the symptoms in older leaves appear as irregular areas of light green tissue.

Commelina diffusa mosaic. This disease, caused by a cucumber mosaic virus, is very common in Hawaii.

Stachytarpheta cayennensis mosaic. This disease of false vervain, characterized by an irregular mosaic pattern in the leaves, was found at Kailua, Oahu, in the papaya ringspot disease area.

Pepper mosaic. This disease is common in Hawaii but the identity of the virus or viruses involved has not been determined.

Crotalaria mosaic. This virus is common on *Crotalaria incana* L. and *C. mucronata* Desv. in the Hawaiian Islands. It causes severe dwarfing of the plant as well as mosaic and malformation of the leaves.

DISCUSSION

Although the papaya is native only to the American tropics (49), during the seventeenth and eighteenth centuries it spread throughout the tropical and sub-tropical regions of the world. As the distribution of papaya was accomplished largely by means of seed rather than by the shipment of growing plants and since no papaya virus has yet been found to be seed-transmitted, it is probable that the virus diseases known to occur in widely separated regions of the world several years ago did not originate in the same locality. The modern plane, however, makes possible the transportation of virus-infected plant parts or of infective insect vectors between all of the papaya-growing areas of the world.

[4] This disease was brought to the attention of the writer by Dr. M. B. Linford of the Pineapple Research Institute of Hawaii.

[5] This disease was brought to the attention of the writer by Dr. R. C. Lindner, formerly of the University of Hawaii, now at Washington State College.

Virus diseases of papaya have been reported from Cuba, Jamaica, the Dominican Republic, Puerto Rico, Tobago, Trinidad, Venezuela, British Guiana, Brazil, Rhodesia, India, Burma, China, Australia, and Hawaii. In addition, a papaya disease characterized by symptoms similar to those caused by some viruses occurs in Java. In 1931 this disease was reported to be due to bacteria. Despite the extreme separation of some of the above regions, several of the described viruses have certain symptoms in common. This is true even in the case of diseases such as bunchy top of Puerto Rico and Waialua disease of Hawaii which are known to be caused by fundamentally distinct viruses. Both diseases typically denude the tree except for a tuft of leaves at the top and both cause water-soaked streaks on the stems and petioles. However, bunchy top is a persistent leafhopper-transmitted virus which is not mechanically transmissible, whereas Waialua disease was readily transmitted by means of plant juice. It is evident, therefore, that the co-identity or close relationship of viruses of papaya from widely separated areas cannot be assumed merely because some of the symptoms are similar. More complete knowledge regarding their vectors and properties as well as their symptoms under various environmental conditions must be acquired before relationships can be inferred.

Some of the symptoms which figure prominently in the descriptions of many known papaya virus diseases are: (1) bunchy top, or the defoliation of the plant except for a few leaves at the top, (2) die-back at the top, (3) curling and distortion of the leaves, (4) water-soaked or oil-like streaks on the petioles and stems, (5) fruit symptoms, and (6) abnormal latex flow when the tree is wounded.

The bunchy top symptom is reported for bunchy top of Puerto Rico, bunchy top (Mosaic type A) and Cotorro mosaic (Mosaic type B) of Cuba, curly leaf of Santo Domingo, yellow crinkle of Australia, mosaic or curl of China, and Waialua disease of Hawaii. In addition, water-logged soil and other adverse environmental conditions may cause a similar symptom.

Die-back is reported to be characteristic of the Trinidad mosaic and also of the die-back disease of Puerto Rico. In addition, Baker (9) speaks of the mosaics of Jamaica and Santo Domingo as die-backs similar to that of Trinidad.

Leaf-curl symptoms and other leaf distortions have been reported from British Guiana, Brazil, Santo Domingo, Cuba (Cotorro mosaic), Puerto Rico (Mosaic), India, Burma, China, and Australia.

Water-soaked areas on the stem, petiole, or both are reported for bunchy top, die-back and mosaic of Puerto Rico, diseases A (bunchy top) and B (Cotorro mosaic) of Cuba, Trinidad mosaic, Waialua mosaic and ringspot of Hawaii.

Bunchy top and die-back of Puerto Rico and bunchy top of Cuba reduce or stop the flow of latex from wounds, while, according to Adsuar (4), in Puerto Rico "this phenomenon is much less common in the mosaic type of disease." Latex flows freely from the wounded tissue of plants in-

fected with Cuban Cotorro mosaic. In Hawaii the latex response of trees carrying ringspot disease is normal whereas, according to Parris (41), Waialua disease causes the fruits to bleed profusely.

Fruit symptoms have been reported for only a few papaya diseases. Yellow spots and rings are caused by ringspot virus in Hawaii and green or dark brown rings are produced by mosaic of Puerto Rico. Spots sometimes appear on the fruit of plants having Cotorro mosaic of Cuba.

The co-identity of any two or more papaya virus diseases must still be established. It is probable, however, that the bunchy top disease of Puerto Rico and type A disease of Cuba are the same. The existence of this disease on other islands of the Caribbean is highly plausible.

Adsuar (4) considers Cotorro mosaic of Cuba to be identical with or closely related to the aphid-transmitted mosaic of Puerto Rico. However, the stem and fruit symptoms reported from Cuba are different from those described from Puerto Rico.

Papaya ringspot disease of Hawaii and papaya mosaic of Puerto Rico resemble each other in that they are both juice-transmissible, both have aphids as their vectors, and in both diseases the time required to produce symptoms is essentially identical. They differ in leaf and fruit symptoms. Ringspot causes a mild-to-marked mosaic mottle in the leaves without conspicuous leaf deformation. The fruit symptoms consist of bright yellow spots and rings at the mature green stage. In Puerto Rico papaya mosaic causes extreme malformation of the leaf and reduction of the lamina to a filiform structure. Green and dark brown rings are produced on the fruit but the stage of fruit maturity at which the rings appear has not been published. The two virus diseases known from Hawaii, Waialua disease and ringspot, are distinct in their reported symptoms.

The reports of papaya virus transmission are relatively few. Mechanical transmission has been announced for Waialua disease and ringspot in Hawaii, mosaic of Puerto Rico, leaf curl of Bihar, India, and curly leaf of Santo Domingo. The latex transmission of curly leaf reported by Ciferri (13) in Santo Domingo needs confirmation. Graft transmission has been reported for leaf crinkle at Coimbatore, South India, and for papaya leaf curl in the Madras Presidency of India.

The only known leafhopper vector of a papaya virus is *Empoasca papaya* Oman which transmits bunchy top in Puerto Rico. Ringspot virus in Hawaii was the first papaya virus to be experimentally transmitted by aphids. Puerto Rico mosaic has also been found to be transmitted by an aphid

In general, virus movement in plants is believed to be associated with the translocation of food. Hence, viruses move rapidly downward in many plants. From the information available in the literature and from an experiment on papaya ringspot it is concluded that downward movement of papaya viruses is relatively slow. The papaya is a rapidly growing, herbaceous, tree-like plant which has no dormancy. As the plant grows the

lower leaves fall and new leaves and flowers are produced at the top. Perhaps the direction of food translocation, therefore, is predominantly between the leaves and fruit in the upper part of the tree with relatively little going from the foliage canopy to the roots. This could reduce the opportunity for virus to become established in the lower part of the plant and might account for the fact that healthy shoots may be produced from the lower parts of papayas infected with viruses such as Trinidad mosaic and Waialua disease. Experiments with papaya ringspot indicate that virus movement upward is rapid in the plant but that downward movement is slow.

SUMMARY

A review is given of the symptoms and transmission reports of papaya viruses recorded from Cuba, Jamaica, Dominican Republic, Puerto Rico, Tobago, Trinidad, Venezuela, British Guiana, Brazil, Rhodesia, India, Burma, China, Australia, and Hawaii.

Papaya ringspot virus, known only from the island of Oahu, T. H., causes a typical mosaic disease in papaya. The virus is carried by aphids and is mechanically transmissible.

Symptoms are produced in the foliage, fruit, and stems of the plant. Rugosity and mottle, without marked distortion, characterize the leaf symptoms. Reduced light enhances the expression of leaf mottle. Yellow spots and yellow rings with green centers are produced on diseased fruits at the mature green stage. The stage of fruit development at the time of infection determines the type of fruit symptom. Fruits infected when young develop yellow rings at maturity while fruits ⅜-grown when infected produce only solid yellow spots. Small, inconspicuous, light green rings sometimes also occur on very young fruits of diseased plants.

Young plants making slow growth when infected sometimes develop water-soaked spots and streaks on the stem.

Primary foliage symptoms usually appear in from 9 to 21 days after infection. Stem symptoms typically take a few days longer to develop than do foliage symptoms.

Plants inoculated in the terminal growth by means of infective aphids develop symptoms a few days earlier than do plants inoculated in the older leaves.

Papaya ringspot virus was shown to move rapidly upward through the papaya plant, but downward movement of the virus was found to be relatively slow.

During experiments to determine the possible host range of papaya ringspot virus, sixteen species of plants representing 12 plant families were inoculated by means of infective aphids. All test plants remained healthy. Non-infective aphids failed to recover virus from any of the 11 plant species used in attempts to recover virus from the symptomless inoculated test plants.

Efforts to transmit 8 virus diseases, occurring in other hosts in Hawaii, to papaya were unsuccessful.

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PAPAYA RINGSPOT VIRUS AND ITS INSECT VECTOR RELATIONSHIPS[1]

D. D. JENSEN[2]

In 1946 and 1947 the writer (2, 3) reported that papaya ringspot virus had been transmitted experimentally in Hawaii by means of *Myzus persicae* (Sulzer) and by three unnamed *Aphis* species. In 1949 (4) experiments were reported, with *M. persicae* as the vector, which dealt with the symptoms and incubation periods of the disease in the foliage, stem, and fruit of the papaya. Data were also presented on the rate of virus movement through the papaya plant and on host range investigations of ringspot virus.

The present paper discusses the relationships found to exist between ringspot virus and *M. persicae*, the most important aphid vector. The identity, host preferences, and vector efficiency are also indicated for other species of aphids shown experimentally to transmit ringspot virus.

MATERIALS AND METHODS

The test plants (solo papaya) and experimental plots used in these investigations were described in a previous paper (4).

Populations of the green peach aphid, *Myzus persicae*, used in most of the vector tests, were reared on pakchoi (*Brassica chinensis*) in the greenhouse. Test insects were usually transferred individually by means of a fine camel's-hair brush. In some of the tests involving winged aphids, forceps were used to pick the insects up by the wings. In many of the tests using 150 or more aphids per test, transfers were made by placing portions of diseased leaves, infested with aphids, in the cage with the healthy test plant. In some of these tests direct contact between diseased and healthy foliage was prevented by placing the inoculum leaf on the soil an inch or two away from the test plant, or by placing the inoculum leaf on a piece of paper which rested on the foliage of the test plant. This precaution was found to be unnecessary, however, because in no instance did ringspot-infected leaves, in the absence of aphids, transmit the virus to healthy plants by contact.

The time of feeding on diseased and healthy test plants varied from 2 minutes to several days depending upon the type of experiment.

Two types of cage were used predominantly in this investigation. In most field tests a single leaf or the growing point of the plant was enclosed in a simple sleeve cage made of white organdy cloth with a weave of 88 threads to the inch. In the greenhouse the sleeve cage was replaced in most tests by a rigid rectangular cage. This was open at the bottom. Three

sides and the top were covered with cloth and one side was covered with clear celluloid. This cage covered the entire plant and the bottom was thrust into the soil around the base of the plant. In a few greenhouse tests, single leaves of small plants were enclosed in sections of transparent sausage-casing, one inch in diameter, cut into sleeves of any desired length.

INSECT SPECIES TESTED AS VECTORS[3]

During the investigation of papaya ringspot disease, 9 species of aphids and one species of spider mite were caged in large numbers on papaya plants (Table 1). *Myzus persicae* was the only aphid species to survive for more than 2 weeks. The other species died out during the first 7 to 10 days on papaya despite the fact that some reproduction occasionally took place.

TABLE 1.—*Summary of papaya ringspot virus vector tests with aphids and red spiders*

Species	Individuals per plant	Number of tests	Number positive	Percentage transmission
Myzus persicae (Sulz.)[a]	100–200	90	76	84[b]
	5	55	10	18
Aphis gossypii Glover	50	10	9	90
	25	3	0	0
	5	10	1	10
Aphis medicaginis Koch	100–150	42	19	45
Aphis rumicis Linn.	100–200	18	14	80
	50–75	15	5	33
	20	2	0	0
Macrosiphum solanifolii (Ashm.)	125	8	2	25
Micromyzus formosanus (Tak.)	200	7	1	14
Amphorophora sonchi (Oest.)	150	6	0	0
Rhopalosiphum pseudobrassicae (Davis)	10	15	0	0
Rhopalosiphum (*Aphis*) *maidis* (Fitch)	50–100	26	0	0
Tetranychus sp., probably *althaeae* v. Hanst	300	18	0	0

[a] The number of tests listed for *Myzus persicae* does not include all of those conducted with the species but represents only those involving high and low numbers of individuals per test.

[b] Of the 14 negative tests, 13 occurred in an experiment involving old inoculum which was a poor virus source.

Some of the other species have been reported to breed on papaya in nature, however, and it is frequently true that insects which sometimes colonize on certain hosts in nature cannot readily be induced to breed by caging them on such plants immediately after removal from a preferred host.

As indicated in table 1, *Myzus persicae, Aphis gossypii* Glover, *A. medicaginis* Koch, and *A. rumicis* Linn. were clearly shown to be vectors of papaya ringspot. *Macrosiphum solanifolii* (Ashm.) with 2 out of 8 tests positive is apparently a vector although more confirmation is needed. *Mi-*

cromyzus formosanus (Tak.) with one out of 7 tests positive may be capable of transmitting the virus, but should be tested more extensively. The number of tests listed for *M. persicae* does not represent all of the experiments involving this species, but includes only those in which either very high or very low numbers of individuals were used per test.

Myzus persicae. Among the insects which breed on papaya in Hawaii, *M. persicae,* the green peach aphid, is found more commonly on papaya than any other insect. Moreover, it greatly surpasses all other species in the density of its populations on papaya. The writer has found several thousand aphids on a few small sucker shoots growing on a single plant. The green peach aphid apparently has little if any toxic effect upon papaya since small plants four inches tall each carried several hundred aphids for a week without marked effect. However, the apical leaves of small, slow-growing sucker shoots produced on the main stem of field trees sometimes are slow to expand when the under surface carries a dense population of aphids. This retardation in growth is probably due to the quantity of plant juice withdrawn by the insects, and not to introduction of a toxin as appears to be the case with *Aphis rumicis.*

Myzus persicae feeds primarily in the veins and veinlets of the leaves. Normally the aphids select the under surface of the leaf but heavy populations frequently extend over the upper surface as well. The tender terminal stem of the young plant is also fed upon. When *M. persicae* was caged on papaya fruits, some of the aphids fed. The feeding punctures in green papaya fruits exuded latex.

The population of *Myzus persicae* in the field in Hawaii fluctuates with the season, weather conditions, and with the abundance of natural enemies. During the cool spring months of 1945 and 1946 the aphids were very abundant on papaya, particularly in the Kailua district. During the warmer, drier summer months the population declined to a relatively low level on papaya although a few heavy colonies could always be found.

The incidence of natural spread of ringspot disease in the papaya plots maintained in Manoa Valley paralleled the field population of the aphid.

In addition to papaya, *Myzus persicae* breeds on a wide range of other cultivated and weed hosts in Hawaii.

Since it is a demonstrated vector of papaya ringspot virus and frequently becomes very abundant on the papaya plant, *Myzus persicae* undoubtedly is responsible for most of the spread of ringspot disease in Hawaii. A few other species of aphids occasionally reproduce on papaya and winged forms of many species are casual visitors to this plant. Some of these species have been found experimentally to be vectors and they are probably responsible for a limited amount of ringspot transmission in nature.

Aphis gossypii. The melon aphid has a wide host range and is encountered more commonly in Hawaii than any other aphid. Holdaway

(1) and Look and McAfee (5) have recorded it as breeding on papaya in Hawaii.

Aphis medicaginis. The bean aphid is common in Hawaii on legumes, *Portulaca oleracea* L., *Amaranthus* sp., and other hosts. Look and McAfee (5) state that it has been observed breeding in abundance on papaya.

The writer has occasionally found apterous *Aphis gossypii* and *A. medicaginis* on papaya in relatively large numbers. However, in each instance the aphid infestation could be traced to preferred breeding host plants which were growing near the papaya tree. In several such cases the recent drying up of the preferred hosts, due to drought or cultivation, had caused a migration of the aphids to nearby green plants including papaya. When caged on papaya the melon aphid and the bean aphid reproduce to a slight extent but the colonies die out within a week or 10 days. Both species were shown to transmit papaya ringspot virus.

Aphis rumicis. During most of the year this aphid is rarely encountered at the lower elevations on Oahu. It can usually be found, however, on fireweed, *Erechtites valerianaefolia* D. C., in the mountains. During March and April, 1946, it occurred abundantly on *Nothopanax guilfoylei* Merr., in Manoa Valley where the papaya ringspot tests were conducted. When caged on papaya, *A. rumicis* survived for 10 days, feeding primarily on the lower surface of the leaves. Approximately 90 percent of the aphids fed in the veins and veinlets of the leaves. Moulting and some reproduction occurred. This aphid produces a toxic effect on papaya in addition to transmitting ringspot virus. After 100 aphids had fed on test trees 18 inches tall for 2 days the 2 youngest partially expanded leaves began to droop and in 3 days they were limp. Experiments summarized in table 1 demonstrated the ability of *A. rumicis* to transmit ringspot virus.

Macrosiphum solanifolii. In Hawaii the potato aphid breeds most abundantly on potato and tomato but it is also reported to reproduce on several other plants including papaya (5). On papaya this aphid survived in cages for 10 days and a few produced progeny. Most of the aphids moved to the underside of the leaves where they fed primarily in the veins and veinlets. Alatae[4] usually survived longer on papaya than did the apterae.[4]

Only 8 ringspot tests were conducted with this aphid. Two of the 8 test trees developed positive though rather mild symptoms. More extensive tests should be carried out with this species.

Micromyzus formosanus. The onion aphid breeds only on onion and chives in Hawaii (5). It was used in 7 ringspot tests to determine whether the virus could be transmitted by species which are unrelated to the more common vectors and which find papaya unacceptable as a host. Transmission occurred in only one case. Although no natural spread occurred among the control plants the work should be confirmed with a longer series before concluding that the onion aphid is a vector of papaya ringspot.

[4] Alatae are aphids which are winged in the adult stage. Apterae are aphids which are wingless in the adult stage.

Amphorophora sonchi (Oestlund). This species breeds commonly only on *Sonchus oleraceus* in Hawaii. It did not feed well on papaya and failed to transmit the virus in the 6 tests in which it was used.

Rhopalosiphum pseudobrassicae (Davis) and Rhopalosiphum (Aphis) maidis (Fitch). Although these aphids fed readily on papaya for a short time they failed to transmit papaya ringspot in these experiments.

Tetranychus sp. probably *althaeae* v. Hanst. The red spider mites of this species are as a rule the most common and must numerous arthropods found on papaya plants in Hawaii. As indicated in table 1, the 18 attempts to transmit papaya ringspot virus with this species were unsuccessful.

VECTOR EFFICIENCY

The efficiency of aphid vectors in transmitting papaya ringspot virus varies considerably within a given species from time to time. Not all of the factors which affect transmission have been established experimentally, but the potency of the virus source is important and is discussed later.

In the tests involving *Myzus persicae,* the most important vector, the number of aphids used per test varied from one individual to several hundred. An indication of the efficiency with which this species transmits ringspot virus can be obtained from a comparison of experiments involving small and large numbers of aphids as presented in table 1. In tests with 5 aphids per plant, transmission was obtained to 10 out of 55 plants, or 18 per cent of the total. In 90 tests with 100 to 200 aphids per plant, 76 plants, or 84 per cent, developed symptoms and 14 failed to acquire the disease. Of the 14 negative tests, however, 13 occurred in an experiment involving old inoculum which proved to be a poor source of virus. As is evident in table 1, 100 or more aphids per plant will usually insure transmission of the disease, providing the aphids have had the opportunity of feeding on a plant carrying virus in relatively high concentration.

FEEDING PERIODS REQUIRED FOR ACQUIRING AND TRANSMITTING VIRUS

Papaya ringspot virus belongs in the group of viruses designated as non-persistent by Watson and Roberts (7) in 1939. Such viruses require no latent period in their insect vectors and are not retained long by their vectors after the latter leave diseased plants.

During this investigation, *Myzus persicae* was shown to acquire ringspot virus from a diseased plant in a feeding time as short as 2 minutes and to infect healthy plants in a feeding period of 5 minutes. Shorter feeding periods were not tested in adequate numbers to determine the minimum feeding time, designated "threshold" by Storey (6) in 1938, necessary to acquire the virus from a diseased plant or to infect a healthy plant.

In experiments summarized in table 2, winged *Myzus persicae* previously starved for 4 or 24 hours were allowed feeding periods of 2 or 5 minutes on diseased leaves. The aphids were then transferred immediately to healthy test plants where they fed for 5 minutes. They were then transferred to

a second and third series of healthy plants. As indicated in table 2, the aphids were able to acquire and transmit ringspot virus in a total time period as short as 7 minutes. Further investigation will probably show that even shorter feeding periods may also result in transmission. These data suggest that the virus has no measurable latent period in the aphid vector.

TABLE 2.—*Loss of papaya ringspot virus by infective Myzus persicae (Sulz.) on the first healthy plant fed upon in serial transfers and proof that aphid vectors may acquire and transmit ringspot virus during short feeding periods*

Number of tests	Number and stage of aphids per plant (1st series)	Fasting period of aphids before acquisition feeding period	Acquisition feeding time on inoculum	Feeding time on 1st test plant	Results +	Results −	Number of aphids to each 2nd healthy plant	Feeding time on 2nd test plant	Results +	Results −
6	200 apterae	0	24 hr.	4 hr.	6	0	50 apterae*	2 days	0	6
5	25 apterae	0	18 hr.	2 days	4	1	10 apterae	2 days	0	5
5	25 apterae	0	18 hr.	2 hr.	4	1	25 apterae	2 days	0	5
7	15 alatae	4 hr.	25 min.	2 hr.	1	6	14 alatae*	2 days	0	7
9	15 alatae	24 hr.	5 min.	5 min.	5	4	15 alatae	5 min.*	0	9
1	15 alatae	4 hr.	2 min.	5 min.	1	0	15 alatae	2 days	0	1
1	10 alatae	4 hr.	2 min.	2 min.	0	1	10 alatae	2 days	0	1
34					21	13			0	34

* Aphids in these tests were allowed a 5-minute feeding period on the second series of plants and were then transferred to a third series where they fed for 2 days. No transmission occurred in the second or third series.

VIRUS RETENTION EXPERIMENTS

Several experiments were conducted in the greenhouse to determine the length of time *Myzus persicae* retains papaya ringspot virus after being transferred from a diseased plant to non-infected hosts.

Large colonies of aphids were transferred from ringspot papaya plants to potato plants upon which they were allowed to feed for periods of time varying from 3 to 21 days. Such aphids transferred from the potato plants to healthy papayas invariably failed to carry ringspot virus.

In a second experiment thousands of aphids, reared on pakchoi, were transferred to ringspot papaya foliage where they were allowed to feed for 24 hours. Aphid-infested ringspot foliage was then cut from the infected plant and laid on pakchoi plants. As the papaya foliage wilted, over a period of 18 hours, the infective aphids crawled to the pakchoi plants where feeding was resumed. Aphid-infested pakchoi leaves were then detached and caged on 15 healthy papaya plants. During the following 12 hours most of the aphids moved to the papaya foliage where they fed. At least 500 aphids fed on each test plant. No infection resulted in any of the papayas used in the experiment.

In a third experiment (Table 2) 200 infective *Myzus persicae* were transferred by brush to each of 6 healthy papaya plants. After feeding for 4 hours on the test plants 50 apterae were transferred from each of the 6 test plants to a second healthy papaya. All of the first 6 plants developed the disease while none of the plants in the second series of 6 became infected.

Table 2 summarizes the results obtained in several additional experiments involving serial transfers of aphids to two or more healthy plants. In some of these tests aphids were forced to fast for a period of 4 or 24 hours prior to feeding on inoculum. In most of such tests the feeding periods for acquiring and transmitting the virus were shortened to 2 or 5 minutes. The results in table 2 demonstrate that in serial transfers, with the transmission feeding time as short as 5 minues per plant, *Myzus persicae* loses the virus on the first healthy plant fed upon. However, if more extensive tests were run with the feeding time of previously fasted aphids reduced to an even shorter period it is probable, in view of the results obtained by Watson and Roberts (8) with other non-persistent viruses, that *M. persicae* could be shown, in a few instances, to transmit the virus to the second or third plant fed upon. Under field conditions in Hawaii, however, it is probable that aphid vectors rarely if ever retain the virus long enough to infect more than a single papaya plant after leaving a ringspot tree.

EFFECT OF VIRUS SOURCE ON INSECT TRANSMISSION

The length of time a papaya plant has been infected with ringspot virus has an effect either upon the concentration of the virus in the plant or upon the availability of the virus to *Myzus persicae*.

Table 3 presents the results of an experiment dealing with this phe-

TABLE 3.—*Influence of virus source on papaya ringspot transmission by Myzus persicae. Approximately 150–200 aphids per test, transferred on pieces of diseased leaves*[a]

Type of inoculum[b]	Number of tests	Positive	Negative	Percentage transmission
Old	7	3	4	43
Old	10	9	1	90
Old	11	3	8	27
Totals for old inoculum	28	15	13	53.6
Young and old	1	1	0	100
Young and old	17	17	0	100
Young and old	6	6	0	100
Young	2	2	0	100
Young	3	3	0	100
Totals for tests including young inoculum	29	29	0	100

[a] Sixty check trees remained disease free.

[b] Old inoculum = young leaves from papaya plants which had been diseased for three months. Young inoculum = young leaves from papaya plants on which symptoms had been evident only 10 days prior to use in this experiment.

nomenon. The insects used in this experiment came from the same stock. They were reared in the greenhouse on pakchoi. The test plants used were 3 to 4 feet in height and were growing under the same field conditions. Although the test feedings took place on leaves of varying maturity among the test plants, both groups included plants inoculated in old and young leaves. Moreover, the data indicate that the age of the leaf into which the aphids inoculated the virus was not a limiting factor. The only apparent effective variable was the length of time the inoculum trees had been diseased.

Inoculum recorded as old was composed of young leaves from plants on which symptoms had been evident for three months prior to use in this experiment. Young inoculum came from plants which had had symptoms for only ten days.

As is evident in table 3, *Myzus persicae* (150–200 aphids per plant) infected 100 per cent of the plants fed upon in all cases in which some or all of the aphids had fed upon young inoculum. In contrast, only 53 per cent of the plants were infected in tests in which only old inoculum had been available to the aphid vectors. Moreover, the results suggest there was considerable variability in the old inoculum from different plants or of different leaves on the same plant. In one series, with old inoculum, 9 of the 10 plants were infected. In another series only 3 out of 8 plants were infected.

FAILURE TO TRANSMIT VIRUS FROM DISEASED FRUITS

Papaya fruits are transported freely in commerce and privately from place to place on the island of Oahu and to a lesser extent between the various islands of the Hawaiian chain. The possibility of infected fruits serving as an adequate virus source for aphid vectors in sections of Oahu, and on islands where papaya ringspot is not known to occur, constituted a potential disease hazard which, if confirmed, might result in a quarantine against papaya fruit movement in the Hawaiian islands.

Under natural conditions *Myzus persicae* feeds primarily on the leaves of the plant. The petioles and main stem of young, tender plants are also attractive feeding sites. In no instance have aphids been found colonized or feeding on fruit in the field. Nevertheless it is possible that occasional feeding on the fruit occurs.

To test the possibility of obtaining transmission from ringspot fruits by means of aphids, large numbers of *Myzus persicae* were confined on infected fruits ranging in maturity from young (one inch in length) to ripe yellow. The aphids were caged on the fruits for from 1 to 2 days. Only those individuals actually observed feeding on the fruits were transferred to the foliage of test plants. Each plant received either 25 or 50 aphids. No transmission was obtained from fruits in 21 tests conducted. These results suggest that aphids rarely, if ever, transmit ringspot virus from infected fruits.

SUMMARY

Myzus persicae (Sulzer), the most common insect on papaya in Hawaii, is the major insect vector of ringspot virus. *Aphis gossypii* Glover, *Aphis medicaginis* Koch, and *Aphis rumicis* Linn. are also shown to be vectors of the disease. *Macrosiphum solanifolii* (Ashm.) and *Micromyzus formosanus* (Tak.) are probably also vectors but the test series with these species were too few to be conclusive.

Myzus persicae acquired the virus in a feeding time of 2 minutes and infected healthy plants in a feeding period of 5 minutes.

There is no demonstrable latent period of the virus in the aphid vector and the virus is not retained by the vector beyond the first healthy plant fed upon after transfer from a diseased plant.

Groups of 150–200 *Myzus persicae* induced infection in 100 per cent of the test plants after transfer from trees showing ringspot symptoms for only 10 days. Similar numbers of aphids transmitted the disease to only 53 per cent of the test plants after transfer from trees which had been diseased for 3 months.

Myzus persicae failed to transmit ringspot virus from infected fruits.

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SOME RELATIONS BETWEEN PHASEOLUS VIRUS 2 AND ITS ASSOCIATED CRYSTALLINE INCLUSIONS

S A U L   R I C H [1]

McWhorter (4) found crystalline inclusions in the nucleoli and cytoplasm of plants infected with Phaseolus virus 2. These inclusions were not found in virus-free specimens. The trypan blue staining technique, first described by McWhorter (3) and later modified by Rich (6), made the detection and observation of these crystals a simple matter. The chemical nature and probable origin of these inclusions have also been described (7). The following paper describes further points of correlation between Phaseolus virus 2 and its associated crystalline inclusions.

SOURCE OF VIRUS

The virus studied was obtained from Blue Lake beans growing at Grand Island, Oregon. The virus proved to be unmixed and produced uniform symptoms through repeated subinoculations.

METHODS

All plant inoculations were made by the carborundum technique (5). The source plant material was ground in a sterile mortar and pestle, and then strained through cheesecloth. The resultant plant extract was applied to the healthy plant leaves with a cotton swab held in sterile forceps. Carborundum powder (600-mesh) was dusted on the leaves to be inoculated prior to swabbing.

All cytological observations were made on unfixed tissues stained with trypan blue.

RELATION BETWEEN THE TIME OF SYMPTOM APPEARANCE AND THE PRODUCTION OF CRYSTALLINE INCLUSIONS

Working with severe etch virus on tobacco, Sheffield (8) found the following results: " . . . rubbed leaves usually showed symptoms within five days, and on the sixth day the veins of younger leaves became cleared. At this time no abnormalities may be seen in either the nuclei or the cytoplasm. On the following day as many as a dozen crystals may be counted in many of the nuclei."

In the present study, broad bean plants (*Vicia faba* L.) inoculated with Phaseolus virus 2 were examined daily from the time of inoculation until after the appearance of symptoms. No crystals were found until the seventh day following the inoculation, before external symptoms were evident. The few small crystals found were only in nucleoli, and stained

weakly with trypan blue.   In the next two days, vein clearing became
evident and the nucleolar inclusions became much larger, more abundant,
and more easily stained.   No cytoplasmic inclusions were present, but they
appeared later.   Cytoplasmic inclusions were produced earliest and in the
greatest abundance in young plants which were growing vigorously at the
time of inoculation.

To summarize, with Phaseolus virus 2 on broad bean, no crystals were
found until a day or two before the first appearance of symptoms, i.e., vein
clearing of the youngest leaflets.   This observation differs from Sheffield's
report in that severe etch inclusions did not appear until two days following
the production of symptoms.

RELATION BETWEEN EXTERNAL SYMPTOM PRESENCE AND THE
PRESENCE OF CRYSTALLINE INCLUSIONS

It has been found in the case of certain systemic plant viruses that
leaves that are mature before the appearance of symptoms remain externally

TABLE 1.—*Distribution of inclusions in broad bean plants just beginning to show
external symptoms of Phaseolus virus 2*

Plant part	External symptoms	Inclusions
Stem growing point	Absent	Absent
Young leaves	Absent	Present
Young leaves	Present	Present
Mature leaves near growing point	Absent	Present
Lowest mature leaves	Absent	Absent
Stem internodes[a]	Absent	Present
Roots	Absent	Absent

[a] Inclusions were also present in the stem between ground level and the node of the
lowest mature leaf.

symptomless (1, p. 86).   This is also true of Phaseolus virus 2 on broad
bean   For this study, plants were chosen which had some leaves with well-
defined symptoms and some without external symptoms.   All leaves on
these plants were examined.   Those externally symptomless leaves nearest
the growing point contained well-developed crystalline inclusions; leaves
nearest the ground level were not only externally symptomless, but also
were devoid of any visible inclusions.   Inclusions were always present in
leaves with symptoms.

Fulton (2) reported the presence of virus inclusions in the roots of
tobacco plants infected with tobacco mosaic   He could find no definite
external symptoms of disease on infected roots.

Broad bean plants having well-developed symptoms of Phaseolus virus 2
on their aerial portions were examined repeatedly for crystalline inclusions
in their roots.   No inclusions were present in roots of infected plants.

Table 1 summarizes the relation between external and internal symptoms
of Phaseolus virus 2.

Inclusions were absent from the root tissues, the growing points of stems, and the oldest leaves. Small crystals were present in the nucleoli of the youngest leaves. The stem, down to the ground level, contained inclusions. Crystals were present in the stem even in the internodes between leaves in which no inclusions were found.

These observations indicate that crystalline inclusions may be induced by Phaseolus virus 2 in parts of broad bean plants without the production of external symptoms.

RELATION BETWEEN PRESENCE OF VIRUS AND PRESENCE OF CRYSTALLINE INCLUSIONS

The following results (Table 2) demonstrate the relation between the presence of recoverable virus and the presence of crystalline inclusions. The inocula were selected from previously examined portions of infected plants. As only five plants were inoculated in each case, additional data are necessary in order to show whether there is no virus in the roots, or whether the root extract decreased the infectivity of the virus. Fulton (2)

TABLE 2.—*Relationship between presence of virus and presence of inclusions*

	Inoculum used		Results of inoculation
Part of plant	Symptoms	Inclusions	
Leaf	Present	Present	Positive
Leaf	Absent	Present	Positive
Leaf	Absent	Absent	Positive
Root	Absent	Absent	Negative

found that tobacco mosaic virus from the roots of host plants was actually less virulent and less resistant to thermal inactivation than was the virus contained in the aerial portions of the plants.

The data presented in table 2 demonstrate that infective virus may be present regardless of whether or not the plant part has either external symptoms or internal inclusions. Crystalline inclusions, however, were found only when the virus was present.

SUMMARY

In broad beans newly infected with Phaseolus virus 2, crystalline inclusions were produced just prior to the first appearance of external symptoms.

Broad bean plants infected with Phaseolus virus 2 may have parts in which no external symptoms appear and yet crystalline inclusions are present.

Virus-infected broad bean plants may have portions that have neither external symptoms nor crystalline inclusions, and yet infective quantities of virus may be obtained therefrom. Crystalline inclusions were found only in plant parts that contained infective quantities of virus.

Broad beans infected with Phaseolus virus 2 showed no root symptoms, nor were crystalline inclusions found in the roots. Virus infection could not be obtained when roots of infected plants were used as inoculum.

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THE VIRUS OF TOBACCO LEAF-CURL

D. G. SHARP[1] AND F. A. WOLF[2]

Tobacco leaf-curl or kroepoek is one of the most serious diseases that affects tobacco and certain other kinds of plants in Venezuela (11). The causal agent of this disease is a virus, and several species of white-flies (Aleyrodidae) have been found to serve as its vectors. Recently, an opportunity was presented for studies on some of the physical characteristics of the leaf-curl virus itself. The virus has been examined in crude sap by means of the electron microscope and a similar investigation has been made on the agent concentrated and partially purified by ultracentrifugation. In addition, some of the sedimentation characteristics of the agent have been determined. The results of these studies and a comparison of the properties of the leaf-curl virus with those of other viruses causing disease in the tobacco plant (1, 2, 3, 6, 7, 8, 9, 13) are reported in the present paper.

GENERAL EXPERIMENTAL PROCEDURES

Although plants affected with leaf-curl were abundantly available in Venezuela, equipment for the contemplated study was not available there. Moreover, importation into the United States of living diseased plants is prohibited. For these reasons the present study was necessarily limited to the use of formolized sap from diseased plants that was sent to the United States. Young leaves of leaf-curl affected plants growing in a field at hacienda Paya, situated near Turmero, in the State of Aragua, were collected and taken to the laboratory of the Departamento de Fitopatología in Maracay. As soon as possible thereafter, the leaves were macerated by passage through a meat grinder. The sap was then pressed out by hand and passed through a coarse cloth that retained only the larger leaf fragments. Formaldehyde was added, as a preservative, in a concentration of 0.1 per cent. The formolized sap was then sent by air-express to the virus laboratories of the Department of Surgery, in the School of Medicine, at Duke University. Here, the material was stored in a refrigerator maintained at 2° to 6° C.

Some of the studies were made on the crude sap. After the crude suspension had stood in the refrigerator for a few days, much of the suspended plant tissue had sedimented leaving a turbid slightly greenish supernatant. Collodion screens for electron micrography of the virus in the sap were

prepared in the usual way, using samples of this supernatant removed with a pipette. The preparations were shadowed with gold. The screens were set at an angle of 11 degrees to the source of evaporating gold or in such a position that the virus particles cast shadows about five times as long as particle height (10).

Concentration and partial purification of the virus were effected in an air-driven quantity ultracentrifuge. A volume of 30 ml. of the supernatant of the sap suspension was spun in an angle centrifuge for 15 minutes at 3,000 g. to remove aggregated material. The nearly clear greenish supernatant was distributed in 2 ultracentrifuge tubes and spun in the air-driven machine for 60 minutes at 25,000 g.
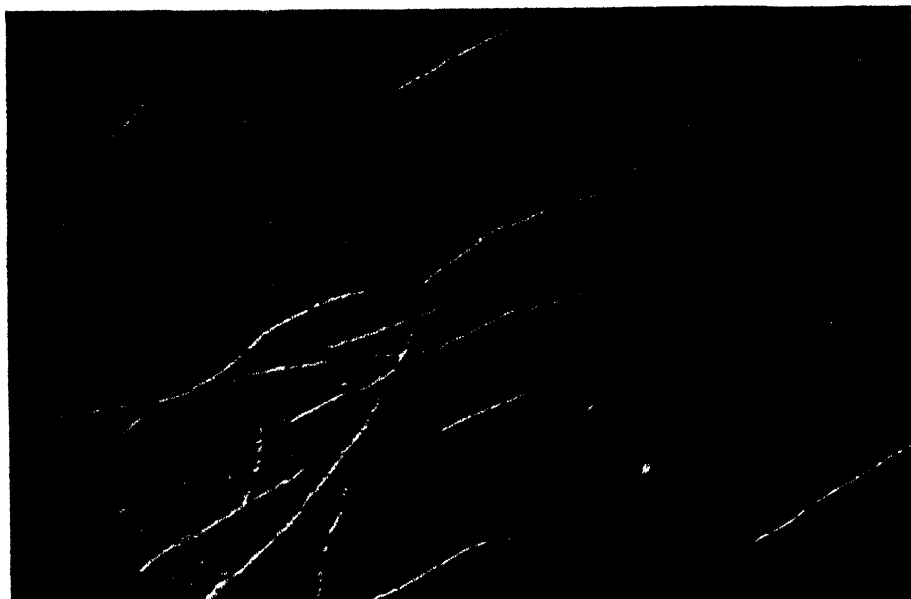
FIG. 1. Electron micrograph of formolized but undiluted crude sap of tobacco plant affected with leaf-curl showing rod-like virus particles. Gold shadow cast at 11 degree angle. Magnification 40,000 ×.

The slightly greenish pellets were resuspended in 15 ml. of 0.1 M phosphate buffer of pH 7.0. This suspension was then centrifuged at 3,000 g. for 15 minutes, and the sediment was discarded. The supernatant fluid was transferred to another tube and was again spun at 25,000 g. for 60 minutes. The large clear pellet that formed was resuspended in 2 ml. of the phosphate buffer, and this suspension is herein regarded as the virus concentrate. Portions of this suspension were prepared for election microscopy as described in the instance of the crude sap.

ELECTRON MICROSCOPY

A representative photograph of the crude sap showing rod-like particles is given in figure 1. The number of these rod-like virus particles in such

preparations is seen to be small and, although their length varies, their width is rather uniform.   Several such preparations were photographed, and the lengths of 67 sharply delimited particles were measured.   These measurements range in length up to 1144 mμ. with a strong maximum at 277 mμ.   The results of the measurements, in histogram form, appear in figure 3, A.

The virus concentrate to be examined was diluted with an equal volume of distilled water.   A representative micrograph of the virus shadowed with gold showing large numbers of the rod-like virus is given in figure 2.   The distribution of lengths from measurement of 200 particles, presented in
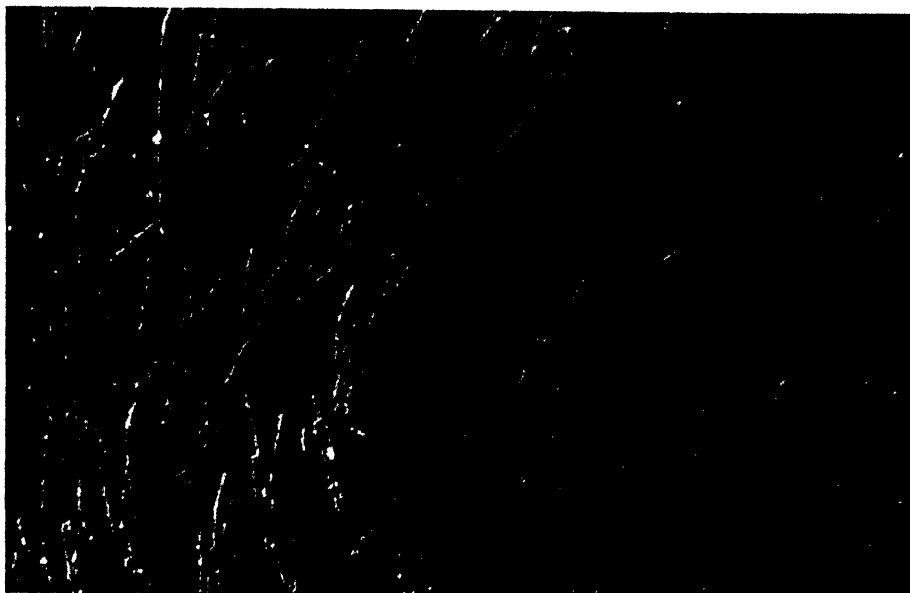
FIG. 2. Electron micrograph of formolized ultracentrifugally concentrated sap of tobacco plant affected with leaf-curl.   Gold shadow cast at 11 degree angle.   Magnification 40,000 x.

histogram form in figure 3, B, shows a strong maximum at 285 mμ.   The width is exceedingly uniform and, when estimated from rods that lie side by side, is about 15 mμ.

SEDIMENTATION VELOCITY STUDIES

The sedimentation velocity of the concentrated virus was measured in an air-driven vacuum type ultracentrifuge carrying a cell 5 mm. thick and 12 mm. high, at a mean turning radius of 6.5 cm.   In the first run, the series of pictures (Fig. 4, A) shows the particles of the concentrate sedimenting as a single exceedingly sharp boundary.   Other runs were made on this material diluted 1: 2, 1: 4, and 1: 8 with 0.1 M phosphate buffer of pH 7.0. As the concentration decreased the boundary became progressively more diffuse.   Figure 4, B shows the boundary obtained with the 1: 8 dilution. In some of the diluted samples a faint secondary boundary could be seen
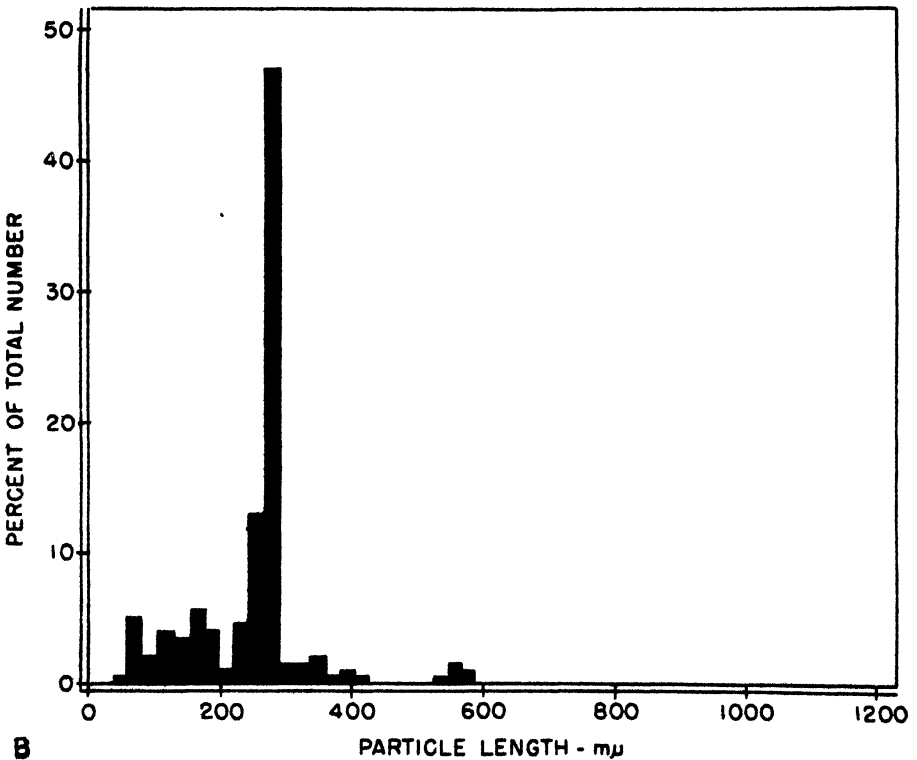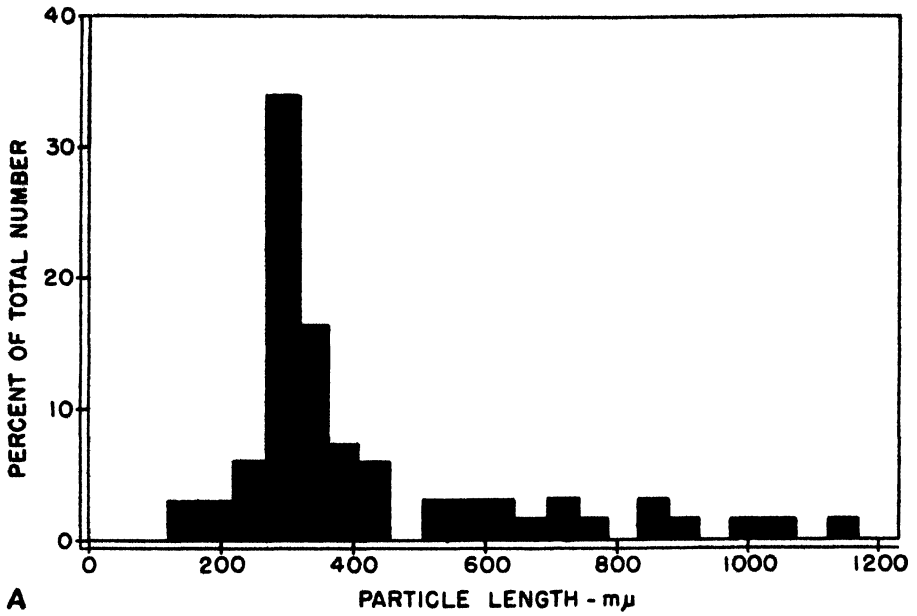
FIG. 3. A. Particle length distribution as seen in electron micrographs of crude material. B. Particle length distribution as seen in ultracentrifugally purified material.

sedimenting faster than the principal one. The sedimentation constants calculated from these four samples in order of dilution were Sw_{20} = 169, 176, 170, and 171×10^{11} c.g.s. units.[3] The faster boundary, seen best in the second (1:2 dilution) run, gave $Sw_{20} = 211 \times 10^{-13}$. Although dilution rendered the boundary more diffuse, it did not change the sedimentation rate as is known to occur for many purified viruses (4).
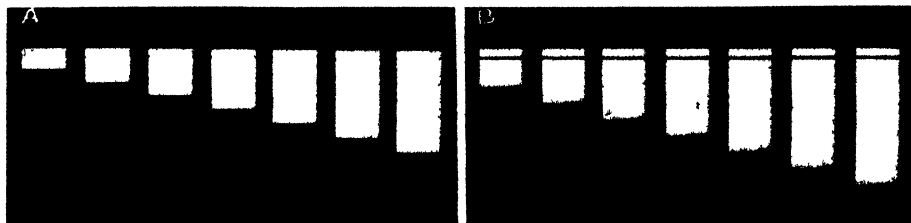
FIG. 4. Sedimentation diagrams of the formalin-treated virus of tobacco leaf curl. a. The concentration is 15 × that in the crude sap and the sedimentation constant calculated from this series of 5-minute-interval pictures at 15,150 R.P.M., is $Sw_{20}o = 169 \times 10^{-13}$. b. The same material diluted 1:8. The time interval and speed are 3 minutes and 20,250 R.P.M. respectively, and the sedimentation constant for the principal boundary below it is $Sw_{20}o = 211 \times 10^{-13}$.

DISCUSSION

The most striking feature found from examination of the formolized sap from leaf-curl-affected tobacco plants is the morphologic similarity of the rod-shaped particles to the virus of ordinary tobacco mosaic. The images of the rods shown in the micrographs herein are identical in appearance with the rods of tobacco mosaic virus in the micrographs of Sigurgeirsson and Stanley (8); moreover, the histograms of length distribution of the two viruses closely resemble each other. The abundance of virus particles in the crude sap of leaf-curl-affected plants is also like that of ordinary mosaic. These similarities are the more remarkable when it is borne in mind that the clinical aspects of tobacco leaf-curl and ordinary mosaic are so different that no one could possibly confuse the two diseases.

Further similarities between the virus of tobacco leaf-curl and that of ordinary mosaic are seen in the sedimentation constants. It is known that under identical laboratory conditions all strains of mosaic virus do not give the same sedimentation constant. The values reported herein for the tobacco leaf-curl virus fall within the range of those in the literature for mosaic virus (13). Even the secondary boundary is like that seen by Wyckoff (12), using three different preparations of tobacco mosaic virus. In one respect only, and that perhaps a minor one, do the sedimentation characteristics of the leaf-curl virus differ from those of the mosaic virus. It is well established that when suspensions of purified viruses are successively diluted the sedimentation rate increases significantly although slowly (4). The range of concentrations used here is sufficient to show such an effect with the leaf-curl virus had it existed to the extent that it does with other viruses.

[3] Sw_{20} is the sedimentation constant reduced to the viscosity of water at 20° C. and expressed in units of centimeters, grams, and seconds (c.g.s.)

It is fully appreciated that biologically active materials may be morphologically indistinguishable yet may induce very dissimilar reactions or responses. Speculation at this time on the underlying reasons for this situation in the case of viruses is quite pointless and meaningless, at least until more is known about the composition and structural configuration of virus proteins.

SUMMARY

The formalin-treated sap of tobacco plants affected with leaf-curl has been studied with the electron microscope. After concentration and partial purification with the ultracentrifuge, further studies were made with the electron microscope, as well as with the analytical ultracentrifuge. All preparations were found to contain rod-like particles, that were regarded as the leaf-curl virus. These particles range in length up to 1140 mμ., but most of them are approximately 280 mμ. long. Their width is uniformly about 15 mμ.

The average sedimentation rate of tobacco leaf-curl virus approximates 171×10^{-13}, which is within the range of variation in rate for ordinary tobacco mosaic virus.

The tobacco leaf-curl virus closely resembles in appearance and in rate of sedimentation certain other viruses that affect tobacco.

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VIRUS CONCENTRATION IN PLANTS ACQUIRING
TOLERANCE TO TOBACCO STREAK

ROBERT W. FULTON

A striking characteristic of the tobacco streak disease, particularly in the field, is the healthy appearance of the leaves which are formed after the development of systemic necrotic symptoms. Johnson (7) has shown that in this respect streak is similar to the ringspot type diseases from which the infected plants have been described by Price (10, 11) as recovering, or acquiring an immunity.

As pointed out by Johnson (7), the tobacco streak disease lends itself to a study of this phenomenon. The differences are distinct between the necrotically affected leaves and the subsequently developed non-necrotic leaves. These non-necrotic leaves, while slightly stunted on greenhouse plants, usually appear healthy in the field. "Skip leaves," or the appearance of necrosis on new leaves above symptomless leaves, as described for tobacco ringspot by McKinney and Clayton (9), do not occur, or occur only rarely on sucker growth.

In a previous publication (6) data were presented which indicated that in infected tobacco both those leaves having systemic necrotic symptoms and the upper, non-necrotic leaves contained a very low concentration of tobacco streak virus when compared with leaves which had been inoculated directly. Price (11) and later Stanley (14) reported that the tobacco ringspot virus was present in considerably higher concentrations in the necrotic leaves as compared to the non-necrotic leaves of infected plants. Since the tobacco streak virus apparently differed from tobacco ringspot virus in the relative concentrations in different tissues, it appeared to merit further investigation.

The presence of an inactivator in extracts from diseased plants also has been investigated, first because of a possible bearing on the recovery phenomenon, and second because prevention of inactivation was necessary for quantitative comparisons of virus concentration.

MATERIALS AND METHODS

Experimental plants were grown in composted soil in 4-inch pots at greenhouse temperatures of 75° to 80° F. For most of the work, the tobacco variety Wisconsin Havana 38 was used. Some comparisons were made using the Turkish variety Xanthia. The tobacco plants were inoculated after 4 to 6 leaves had developed. Samples for assay were taken after 3 or 4 symptomless leaves had developed above the systemically necrotic leaves.

The tobacco streak virus was a type commonly found in the field. The strain used was obtained from one local lesion on Great Northern bean inoculated with a field collection of virus. Since other strains have been isolated which differ in severity of symptoms produced, it seemed advisable to confine the work to a single strain.

Estimates of tobacco streak virus concentration were made by counting local lesions appearing on inoculated leaves of guar (*Cyamopsis tetragonalobus* (L.) Taub.). In comparing virus concentrations, usually 3 different preparations of inoculum from one source were applied to the left or right halves of 9 leaves on 3 guar plants. The comparable preparations from the other source were inoculated to the opposite half-leaves. Whenever possible, the preparations were arranged in a randomized latin-square design on the assay plants. When it was necessary to make comparisons between 3 or more sources or treatments, these were either paired on opposite half-leaves, or one treatment was selected as a standard and the others compared with it on opposite half-leaves. All inoculations were made on leaves lightly dusted with carborundum powder.

Because previous work (6) had indicated a rapid loss of infectivity in the expressed juice of streak-infected tobacco leaves, inoculations were made as rapidly as possible after grinding the tissue. The common method of making serial dilutions of expressed juice could not be used, because even the short interval between grinding the tissue and the final inoculation resulted in considerable loss of infectivity. Rather than serial dilutions, therefore, inocula containing different proportions of tissue were used. Disks were cut from lamina of infected leaves with a sterile 5/16-inch cork borer. The fresh weight of 100 such disks was about 1 gram. In preparing inocula, each disk was considered equivalent to 0.01 ml. of expressed juice. Ten disks, ground in 0.4 ml. of phosphate buffer at pH 7.0, are referred to as a tissue concentration of 1:5; and 10 disks in 2.4 ml as a tissue concentration of 1:25. For the more dilute inocula, the tissue was ground in a small amount of buffer and the remainder pipetted into the mortar after grinding. Comparisons were thus made between equal areas of infected leaf tissues rather than between equal volumes of expressed sap.

In assaying the concentration of the tobacco ringspot virus, the inoculum was prepared in the same manner as with the tobacco streak virus. In most cases, the lesions produced on primary leaves of young cowpea plants were counted. When it was found that the tobacco ringspot virus also produced distinctive local lesions on guar, this plant was used in some trials.

In this paper, the term "acquired tolerance" is used to refer to the development of healthy appearing leaves which follow the appearance of necrotic symptoms of tobacco streak. For convenience, these upper, non-necrotic leaves of infected plants are referred to as "tolerant leaves," and leaves with systemic necrotic symptoms are referred to as "necrotic leaves."

EXPERIMENTAL RESULTS

The Presence of an Inactivator in Leaf Extracts

In preliminary comparisons of virus concentration in necrotic and tolerant leaves, it was found that dilution resulted in an increase in infectivity. The infectivities of four concentrations of necrotic tobacco leaf tissue, 1:1,

1:5, 1:25, and 1:125, were compared on opposite half-leaves of guar with the same concentrations of tolerant leaf tissue. In three trials, the average numbers of lesions obtained with tissue from necrotic leaves, in decreasing order of concentration, were 60, 96, 110, and 41. The figures for comparable inocula from tolerant leaves, in the same order, were 29, 61, 163, and 105. Only a small number of lesions was produced by the most concentrated inoculum, while the greatest infectivity was obtained with tissue concentrations of 1:25.

The increase in infectivity of the preparations with increasing dilution is similar to the reactivation of inactive virus in mixtures with certain inactivators, as has been described by Kuntz and Walker (8). The results suggested that the infected tissue contained some inactivator, and it seemed possible that such an inactivator might be related to the recovery phenomenon.

TABLE 1.—*The effect of extracts of healthy tobacco leaf tissue on the infectivity of a 1:25 tissue concentration of the tobacco streak virus. Treated inocula contained 20 per cent healthy leaf tissue*

Method of preparing inoculum	Total lesions on 6 half-leaves of guar in each of 5 trials				
	I	II	III	IV	V
Healthy tissue ground with infected tissue and buffer immediately before inoculation	306	182	66	491	766
Healthy tissue ground in buffer 20 minutes before grinding with infected tissue and inoculating	7	5	8	84	8
No healthy tissue added	453	327	112	631	712

Diachun (5) has shown that extracts of healthy tobacco leaves will inactivate the tobacco streak virus. Preliminary attempts to confirm his results were occasionally unsuccessful. The variations were found to be due to differences in the time that the healthy extracts were exposed to air before mixing with virus. In one series of trials, inoculum was prepared containing 1:25 tissue concentration of tobacco streak virus and 20 per cent healthy tobacco leaf tissue. The effect of grinding both tissues together and inoculating immediately was compared with the effect obtained when the healthy leaf tissue was ground 20 minutes before grinding the infected tissue and inoculating. The results of 5 trials are presented in table 1. Healthy tissue, in most trials, had a slight inactivating effect. After being ground and exposed to air for 20 minutes, the inactivating effect was pronounced.

It seemed likely that the reactivating effect obtained by diluting infected tissue might be related also to the formation of an inactivator in the crushed infected tissue. To test this possibility, inocula from a single infected tobacco leaf were prepared in 3 concentrations, 1:1, 1:5, and 1:25.

Comparisons of the infectivities of the preparations were made immediately after grinding, and after 5, 10, and 20 minutes. The results of one trial are shown in figure 1, and were typical of other trials. The infectivity of the 1:1 preparation decreased very rapidly; that of the 1:5 preparation somewhat less rapidly. The 1:25 preparation lost no infectivity within 20 minutes. At the end of 20 minutes, the 1:25 preparation was the most infectious and the 1:1 preparation the least infectious.

It thus appeared that the low infectivity of the higher concentrations of inoculum in the preliminary trials was due to the formation of an inacti-
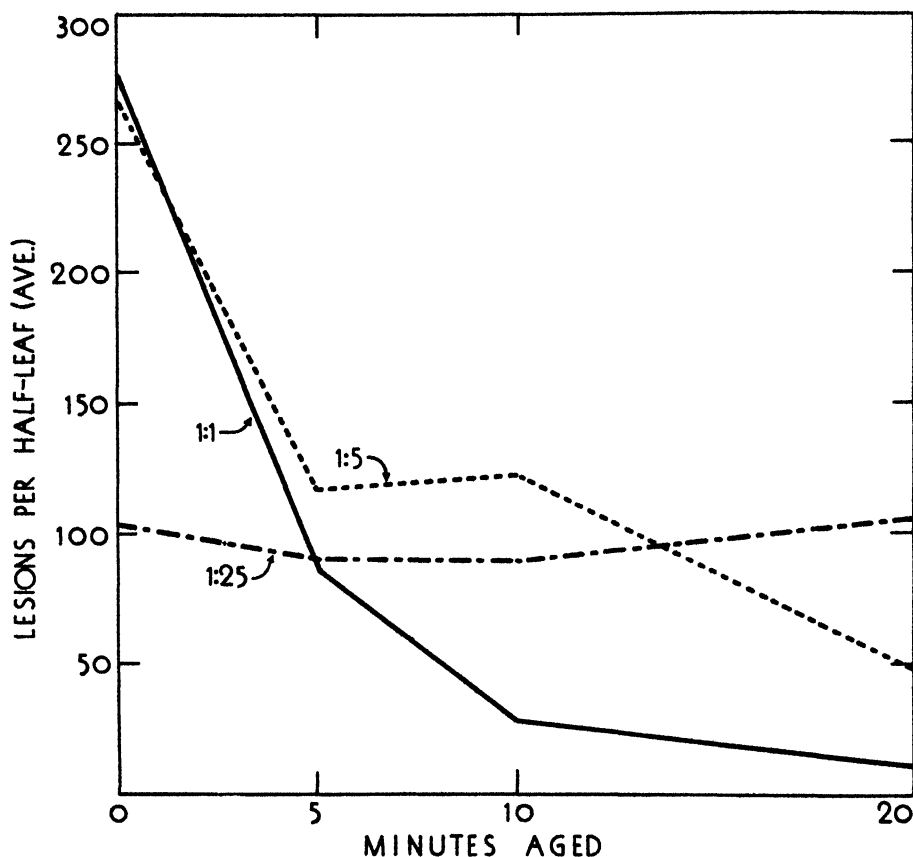
FIG. 1. The effect of the concentration of inoculum on the rate of inactivation of the tobacco streak virus by aging.

vator in the crushed tissue, in spite of attempts to make inoculations as rapidly as possible. Apparently in the more dilute inocula the concentration of inactivator formed was too low to have a detectable effect.

The primary cause of the rapid "aging" of the virus in extracts was the formation of apparently the same inactivator as appeared when healthy tissue was crushed and aged. In an attempt to determine whether both types of inactivation were due to the same cause, the relation of the age

of the infected leaf to the rate of aging of the virus was determined.   In
one trial, tissue from aiT inoculated upper tobacco leaf, diluted with 4
parts of buffer, gave an average of 246 lesions per half-leaf of guar when
inoculated immediately, and an average of only 5.5 lesions after standing
20 minutes.   The same concentration of inoculum from an inoculated lower
leaf of the same plant produced an average of 272 lesions when inoculated
immediately, and an average of 166 lesions after standing 20 minutes.
The results of other trials were similar, except in a few instances, when
inoculum from lower leaves lost infectivity nearly as rapidly as that from
upper leaves.

Determinations were then made of the inactivating effect of fresh and
aged healthy tissue from upper and lower tobacco leaves comparable to
the infected leaves used in the preceding trials.   A 1:25 preparation of
virus was treated either by grinding with a total of 20 per cent healthy
tissue and inoculating immediately, or by grinding the healthy tissue
first, allowing it to stand for 20 minutes, and then grinding the in-
fected tissue with this pulp and inoculating   In one trial the unaged
healthy tissue from an upper leaf inactivated 32 per cent of the virus, as
compared to the control containing no healthy tissue.   The aged extract
from the same leaf inactivated 98.5 per cent of the virus.   The unaged
extract from the lower leaf caused no inactivation, while after aging 20
minutes it inactivated 62 per cent of the virus.   Other trials gave similar
results.   The increased rate of aging of the virus in extracts of younger
leaves was correlated with the increased rate of formation of inactivator
in extracts of younger, healthy leaves of the same size and age.

Since the inactivator appeared in extracts after the tissue had been
crushed and exposed to air, and since it appeared to approximately the
same extent in extracts of both healthy and diseased leaves, it is not likely
that it is related to the phenomenon of acquired tolerance.   It was further
found that rapid aging of the tobacco streak virus occurs in extracts of
infected *Nicotiana glutinosa* and *N. rustica*, plants which do not react to
the virus in the same manner as *N. tabacum*.

Relation of Oxidation to the Formation of Inactivator

The evidence suggested that the inactivating substance appearing in
extracts of tobacco leaves was the result of an oxidation.   To test this pos-
sibility, apparatus was devised by which tissue could be crushed in a per-
forated test tube which was sealed within a small flask by rubber connec-
tions.   Before crushing the tissue, the oxygen in the flask was exhausted
by an alkaline solution of pyrogallol.   In every trial, crushed tissue held
for 20 minutes in an oxygen-free atmosphere lost little or no infectivity.
In control experiments, omitting only the pyrogallol, extracts lost 96 to 99
per cent of their infectivity within 20 minutes.

The results are similar to results described by Bald and Samuel (2)

and Best and Samuel (4) with the tomato spotted wilt virus. These investigators, and later Best (3), also showed that the presence of reducing agents in the inoculum had a preservative effect on the virus of tomato spotted wilt. Similarly, Ainsworth and Ogilvie (1) showed that lettuce mosaic was more infectious and could be preserved longer when sodium sulphite was added to the inoculum. Because the rate of inactivation of the tobacco streak virus was not the same in all preparations, some method of preventing the inactivation was necessary in making quantitative comparisons. Several reducing agents were tested for this purpose. One part

TABLE 2.—*The preservative effect of certain reducing agents on the tobacco streak virus in preparations of 1 part of infected tissue ground in 4 parts of phosphate buffer (pH 7.0) containing the reducing agent*

Reducing agent	Trial	Average lesions per half-leaf of guar produced by preparations:			
		Containing reducing agent		Without reducing agent	
		Unaged	*Aged 20 min.*	*Unaged*	*Aged 20 min.*
Cysteine hydrochloride (0.01 molar)	I	364	395	119	24
	II	73	69	131	2
	III	211	172	199	2
Glutathione (0.01 molar)	I	354	309	77	17
	II	136	16	139	2
	III	138	155	208	3
Sodium thioglycollate (0.01 molar)	I	265	159	58	9
	II	64	59	55	1
	III	187	193	204	5
Potassium cyanide (0.01 molar)	I	251	19	141	8
	II	84	5	130	5
	III	336	28	296	22
Sodium sulphite (0.01 molar)	I	465	371	148	26
	II	146	89	159	9
	III	127	144	172	14
Iron filings (40 mg. per ml.)	I	299	149	87	14
	II	153	108	123	3
	III	278	92	289	11

of infected tobacco leaf tissue was ground in 4 parts of 0.01 molar solutions (or 40 mg. per ml. of iron filings) of the reducing agent in pH 7 phosphate buffer. Inoculations with this preparation were made immediately, and after 20 minutes, to half-leaves of guar. As controls, similar preparations containing no added reducing agent were inoculated immediately, and after 20 minutes, to the opposite half-leaves of the test plants. The results of 3 trials with 6 reducing agents are presented in table 2.

In the presence of 0.01 M concentrations of cysteine hydrochloride, glutathione, sodium thioglycollate, sodium sulphite, and 40 mg. per ml. of iron filings, the infectivity of the preparations remained nearly constant for 20 minutes, or decreased less than did the infectivity of control prepa-

rations.   In all trials, inoculum without an added reducing agent gave very few lesions after being aged 20 minutes.

For each of the 3 trials, all the inoculum was taken from a single infected tobacco leaf in an effort to minimize any differences in the tissue to be treated by different reducing agents.   In trial I, the presence of each of the 6 substances resulted in a considerably greater number of lesions than was produced by the control inoculum, even though the control was inoculated within 1 minute after grinding the tissue.   This effect was produced by potassium cyanide, although it had little or no preservative effect on the virus.   Either a reactivation of virus occurred in the treated extracts, or a very rapid inactivation of virus occurred in the control extracts.   Further trials showed that, when cysteine hydrochloride was added to inoculum which had been ground and aged 20 minutes, there was no increase in infectivity as compared to the aged control.   It was concluded that once the virus was inactivated it could not be reactivated by a reducing agent.   Apparently, also, an appreciable inactivation of virus may occur within a very short time in some extracts.

Virus Concentration in Relation to Acquired Tolerance

After it was found that the rapid inactivation of the tobacco streak virus in extracts could be prevented by including a reducing agent in the inoculum, another series of comparisons was made of virus concentration in necrotic and tolerant leaves.   The experimental procedure was the same as had been used previously, except that the buffer in which the disks were ground contained 0.01 molar cysteine hydrochloride, freshly dissolved. The infectivity of 3 tissue concentrations from necrotic leaves was com-

TABLE 3.—*The relative concentration of tobacco streak virus in necrotic and tolerant leaves of Havana 38 tobacco.   All inoculum contained 0.01 molar cysteine hydrochloride*

Tissue concentration	Source of inoculum	Total lesions on 3 half-leaves of guar in each of 6 trials					
		I	II	III	IV	V	VI
1:5	Necrotic leaves	554	313	491	313	370	484
	Tolerant leaves	633	569	832	257	418	867
1:25	Necrotic leaves	555	88	340	138	233	316
	Tolerant leaves	641	385	689	136	288	606
1:125	Necrotic leaves	108	5	5	10	15	33
	Tolerant leaves	290	68	180	37	93	196
Ratio of infectivities, Necrotic/tolerant[a]	Log ratio and semi-confidence interval (t 0.05)	−0.23 ±0.20	−0.52 ±0.22	−0.17 ±0.22	−0.07 ±0.07	−0.34 ±0.19	−0.23 ±0.20
	Ratio	0.58	0.30	0.67	0.85	0.46	0.58

[a] The method of calculation was that described by Price and Spencer (12).

pared on opposite half-leaves of guar with the infectivity of the same 3 tissue concentrations from tolerant leaves. The results are presented in table 3.

In comparing the infectivities of the tissues, the ratio of the infectivity of necrotic tissue to the infectivity of tolerant tissue was determined. The log of this ratio and its semi-confidence interval (standard error multiplied by t 0.05) were calculated by the method described by Price and Spencer (12).[1] In all cases the ratio (antilog) was less than 1. The tolerant tissue evidently contained more active virus per unit of area than did the necrotic tissue. This may have been due partly to the fact that a small proportion of the total area of tissue from necrotic leaves was composed of dead cells, which presumably contained no virus. The tissue from tolerant leaves, on the other hand, showed no observable necrotic areas.

TABLE 4.—*The relative concentration of tobacco ringspot virus in necrotic and tolerant leaves of Havana 38 tobacco*

Tissue concentra- tion	Source of inoculum	Total lesions on 2 half-leaves of cowpea in each of 7 trials						
		I	II	III	IV	V	VI	VII
1 : 250	Necrotic leaves	302	137	294	406	378	409	274
	Tolerant leaves	303	163	453	425	364	411	307
1 : 1250	Necrotic leaves	68	20	63	94	147	111	132
	Tolerant leaves	57	30	100	153	138	115	161
1 : 6250	Necrotic leaves	6	2	3	11	16	11	4
	Tolerant leaves	4	3	16	18	16	10	7
Ratio of in- fectivities, Necrotic/ tolerant[a]	Log ratio and semi confidence interval (t 0.05)	.039 ±.017	−.054 ±.024	−.209 ±.143	−.184 ±.049	.037 ±.133	.007 ±.016	−.044 ±.028
	Ratio	1.09	0.88	0.62	0.65	1.09	1.00	0.90

[a] The method of calculation was that described by Price and Spencer (12).

The results do not show a lower concentration of tobacco streak virus in tolerant leaves, and they differ from those of Price (11) on the tobacco ringspot virus in this respect. His comparisons, also made between equal areas of different leaves, showed that the concentration of tobacco ringspot virus was considerably lower in tolerant leaves than in necrotic leaves. Since his determinations probably were made under somewhat different conditions, they were repeated, using ringspot-infected Havana 38 tobacco plants grown under the same conditions as the streak-infected plants. Tissue samples were taken with a sterile cork borer, and the inocula prepared as with tobacco streak. Since it appeared possible that the presence of a reducing agent might increase infectivity, several trials were run to determine the effect of 0.01 molar cysteine hydrochloride on the tobacco

ringspot virus. No significant difference in infectivity was found between extracts with and those without this reducing agent. There was no detectable loss of infectivity of tobacco ringspot virus extracts, without the reducing agent, within 20 minutes. Consequently, inocula were prepared with only pH 7 phosphate buffer. Three tissue concentrations, 1:250, 1:1250, and 1:6250, from necrotic and tolerant leaves were applied to opposite halves of primary cowpea leaves. Three cowpea plants, providing

TABLE 5.—*The relative concentrations of tobacco streak and tobacco ringspot viruses in necrotic and tolerant leaves of Xanthia tobacco. The tobacco streak inoculum contained 0.01 molar cysteine hydrochloride*

Tobacco streak virus		Total lesions on 3 half-leaves of guar in each of 5 trials				
Tissue concentration	Source of inoculum	I	II	III	IV	V
1:5	Necrotic leaves	494	300	320	99	109
	Tolerant leaves	507	297	608	378	120
1:25	Necrotic leaves	236	230	149	18	11
	Tolerant leaves	255	237	374	75	25
1:125	Necrotic leaves	54	31	3	0	0
	Tolerant leaves	64	44	16	6	2
Ratio of infectivities, Necrotic/tolerant[a]	Log ratio and semi confidence interval (t 0.05)	−0.034 ±0.02	−0.057 ±0.046	−0.221 ±0.089	−0.291 ±0.106	−0.100 ±0.041
	Ratio	0.92	0.87	0.60	0.51	0.79
Tobacco ringspot virus		Total lesions on 2 half-leaves of cowpea				
1:250	Necrotic leaves	329	430	267	336	294
	Tolerant leaves	130	220	131	157	48
1:1250	Necrotic leaves	75	65	55	103	50
	Tolerant leaves	9	19	12	34	5
1:6250	Necrotic leaves	7	5	4	12	3
	Tolerant leaves	0	2	0	3	0
Ratio of infectivities, Necrotic/tolerant[a]	Log ratio and semi-confidence interval (t 0.05)	0.365 ±0.076	0.191 ±0.063	0.208 ±0 060	0.323 ±0.054	0.379 ±0.101
	Ratio	2.32	1.55	1.61	2.10	2.39

[a] The method of calculation was that described by Price and Spencer (12).

a total of 12 half-leaves, were used in each trial. The results of 7 trials are presented in table 4.

The infectivity of tobacco ringspot virus extracts from tolerant leaves was at least as great as that from necrotic leaves. Similar results were obtained in a series of comparisons using guar as an assay plant. The results were consistent with those on the tobacco streak virus in indicating that tolerant leaves contained as much or more virus than necrotic leaves.

A factor which might have contributed to the difference between the results in this investigation and those of Price (11) was the use of differ-

ent types of tobacco. Since his studies were made on Turkish tobacco, additional trials were made with each virus, using infected plants of the Turkish variety Xanthia. The results are presented in table 5.

In contrast to the results with Havana 38 tobacco, the concentration of the tobacco ringspot virus appeared considerably lower in the tolerant leaves of the Turkish tobacco, as was reported by Price (11). With the tobacco streak virus, on the other hand, the concentration of virus in the tolerant leaves was slightly greater than in the necrotic leaves.

From the foregoing results it was concluded that a low virus content is not correlated with the absence of necrosis of the uppermost leaves of plants infected with the two viruses. Although the new upper leaves display only slight symptoms, apparently multiplication of the viruses in them may be unrestricted.

Virulence of the Virus in Tolerant Leaves

Since the symptomless condition of the upper leaves of streak-infected tobacco could not be explained by any restriction imposed by the plant upon multiplication of the virus, another possibility was considered. If a mild strain of the virus should appear in infected plants and move rapidly into the growing tip, it might prevent the invasion or production of symptoms by the more severe strain of virus. To test this possibility, material from tolerant leaves of streak-infected tobacco was inoculated to the primary leaves of Great Northern bean. The local lesions which developed were removed separately with a sterile cork borer and inoculated to healthy tobacco. A total of 148 single lesions produced the same necrotic symptoms characteristic of the original disease. In these trials, three strains of tobacco streak were used, distinguishable from each other by the type and severity of the necrotic symptoms. The single-lesion isolates from tolerant leaves in every case were identical to the strain of virus originally infecting the plant. It was concluded that no change in virulence of the virus had occurred in the tolerant leaves.

DISCUSSION

The investigation was undertaken with the hope of adding information to the subject of acquired tolerance to virus diseases in plants. The tobacco streak virus seemed especially suitable for such a study. Comparisons also were made with the tobacco ringspot virus, particularly with regard to relative concentration of virus in different tissues. The sequence of symptoms of plants infected with each of the viruses is similar. With the strain of tobacco streak virus used, the "recovery" of infected plants is more rapid and more complete than with the tobacco ringspot virus. The data, however, showed no fundamental differences in the behavior of each virus within the host. The properties of the two viruses, on the other hand, are not similar so far as is known. It seems possible that a

closer comparison might reveal certain properties which are similar and which might have a bearing on the similar reaction of plants infected with these viruses.

The presence of tobacco ringspot virus in relatively low concentration in tolerant leaves of Turkish tobacco, and in high concentration in tolerant leaves of Havana tobacco, could not be correlated with any visible difference in the reaction of the plants to the virus. It does not seem probable that the low concentration of virus is directly related to the absence of symptoms in one variety and not in the other. It seems more likely that the low concentration in tolerant leaves of Turkish tobacco is not causally related to the type of symptom.

The data obtained show that the tobacco streak virus is present in the symptomless leaves of infected plants in undiminished concentration and virulence. What appears to be a change in the reaction of the plant to the virus occurs between the initial infection and the development of new leaves following the systemic movement of the virus. This change, however, may be due to a change in the location or distribution of the virus. Price (11) has suggested that embryonic cells of the stem tip become adapted to the presence of the tobacco ringspot virus, and are able to develop nearly normally in spite of the virus. Valleau (15) has postulated a more or less complete invasion of the embryonic tissue by the virus, and that the subsequent formation of new leaves uniformly invaded by the virus does not result in the line and ring type of symptom. In the present investigation some trials were made which, while not conclusive, indicated that the embryonic tissue in the stem tips of infected plants contained as much virus per unit weight as any other part of the plant. Since the necrotic lines characteristic of tobacco streak seem to develop near the advancing margin of the virus as it invades a leaf, it is possible that young leaves formed with a uniform distribution of virus within them would react differently.

McKinney and Clayton (9) have interpreted the development of the "chronic phase" of ringspot as being due to a high degree of resistance to necrosis in the young leaves at the time they are invaded. In streak-infected tobacco, also, the youngest bud leaves do not become necrotic when systemic symptoms develop on older leaves. Almost invariably the youngest leaf to show symptoms will be necrotic only at its tip, the most nearly mature portion. Whether the bud leaves continue to grow without becoming necrotic because they retain this resistance, or because they are subject to a different invasion pattern of virus, or for some other reason, cannot be answered on the basis of the present data.

The terms which have been used to describe the development of non-necrotic leaves on ringspot-infected plants have been based on different interpretations of experimental results. Price (10) applied the term "acquired immunity" to plants infected with the tobacco ringspot virus be-

cause they produced symptomless leaves which did not become necrotic when reinoculated. Since such leaves are immune from a set of symptoms and not from the virus, objections have been raised to the application of the word "immunity." Similar objections have been raised to the use of the term "recovery." Valleau (15) and McKinney and Clayton (9) would prefer to use the term "chronic disease."

The term acquired immunity has been applied also to protective inoculation, including cases where infection with the protecting strain of virus does not lead to the appearance of symptomless leaves. This protection or immunization appears to depend chiefly upon complete invasion by the protecting virus. That symptomless leaves of ringspot or streak-infected plants are protected against reinoculation is apparent. That this protection is the cause of the development of symptomless leaves has not been demonstrated. It is likely that immunity to reinoculation is caused by factors different from those which lead to the development of symptomless leaves. It would seem better, therefore, to use separate terms which would express more precisely the intended meaning.

In this paper the term "acquired tolerance," as suggested by Smith (13), has been used because it most adequately expresses the actual condition of the infected plant. The term "chronic disease" might also be used, but it was felt that this did not sufficiently emphasize the symptomless condition which is so characteristic of the tobacco streak disease.

The formation of an inactivator of the tobacco streak virus by oxidation of some component in extracts of healthy or diseased tobacco presents some interesting possibilities for further study. Susceptibility to the same type of inactivator as affects the tomato spotted wilt virus suggests that there may be other viruses equally, or more, susceptible to the same type of inactivator. The addition of reducing agents to inoculum might permit mechanical transmission in some cases where otherwise it is difficult or impossible.

SUMMARY

When attempts were made to compare the concentration of tobacco streak virus in necrotic leaves with the concentration in symptomless new leaves, it was found that infectivity increased with increasing dilution. This reversal of the normal dilution curve was found to be due to the formation of an inactivator when extracts of diseased or healthy tobacco leaves were exposed to air. Because the inactivator appeared only after crushing the tissue, and because healthy and diseased leaves were equally good sources, it was concluded to be unrelated to the phenomenon of acquired tolerance.

By adding reducing agents (cysteine hydrochloride, glutathione, sodium thioglycollate, sodium sulphite, iron filings) to the inoculum at the time the tissue was ground, rapid inactivation of the virus was prevented. Untreated inoculum lost 75 to 99 per cent of its infectivity within 20 minutes.

When infected tissue for assay was ground in the presence of a reducing agent, the concentration of tobacco streak virus was found to be as high, or higher, in tolerant leaves as in necrotic leaves of both Havana 38 and Xanthia varieties of tobacco. The concentration of the tobacco ringspot virus in infected Havana 38 tobacco was approximately the same in tolerant and necrotic leaves. In Xanthia tobacco, there was a higher concentration of this virus in necrotic leaves than in tolerant leaves.

Single lesion isolations of tobacco streak virus were made from bean leaves inoculated with tolerant tobacco leaves. The single lesion strains from tolerant leaves were as virulent as those from necrotic leaves.

It was concluded that the production of new symptomless leaves on plants infected with the tobacco streak virus is not due to any reduction in the amount of virus or in the virulence of the virus present.

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MODIFICATIONS OF THE SLIDE-GERMINATION METHOD OF EVALUATING FUNGICIDES INCLUDING THE USE OF VENTURIA INAEQUALIS AND PHYTOPHTHORA INFESTANS[1]

HAROLD J. MILLER

Reddick and Wallace (34) first pointed out that the method of using a spore suspension on dried spray deposit "more nearly simulates natural conditions than that of using a drop of the spray substance direct". Attempts have been made to use a dried deposit on slides but usually such an elaborate apparatus is required that it has been more practical to do the preliminary testing directly in the drop of spray suspension without any drying. A simplified method was developed in this study for using a dried spray film. Procedures were developed for growing and using the spores of the apple scab organism (*Venturia inaequalis* (Cke.) Wint.) and potato late blight organism (*Phytophthora infestans* Mont. DeBary); in addition, the techniques for using the brown rot organism (*Sclerotinia fructicola* (Wint.) Rehm.) were modified. Attention was also given to a study of the role of certain materials in spore germination and dosage-response with these organisms as well as with *Alternaria oleracea* Milbraith (18).

GENERAL TECHNIQUE

Surface. Glass slides have been used extensively and are recommended (1) for this type of test. However, as pointed out by Horsfall et al. (12), drops of water do not spread uniformly on glass surfaces. They described a method of coating the slides with cellulose nitrate which gave drops of uniform diameter (7.5 mm.). Cellulose nitrate sheets sold under the name of Pyralin (9) give drops of the same size as the coated glass slides. The sheets can be easily cut to the standard size (3 × 1 in.).

A comparison of check slides using *Sclerotinia fructicola*, *Venturia inaequalis*, and *Phytophthora infestans* showed no significant difference in spore germination between glass, glass coated with cellulose nitrate, and Pyralin surfaces (Table 1). Tenacity coefficients as determined by spore germination on spray deposits made according to the method of Frear (9) before and after weathering were not significantly different on these three surfaces (Table 1).

Application of standard deposit. As pointed out by McCallan and Wilcoxon (22) the difficulty involved in obtaining a known uniform deposit

245

is one of the principal sources of error in the laboratory assay of fungicides. After critically testing several methods for uniformity of deposit, McCallan and Wilcoxon (22) recommended a settling tower. Horsfall et al. (12) described a horizontal sprayer for this purpose. Montgomery and Moore (29, 30) and Young (40) avoided the use of a spray apparatus by pipetting measured amounts of suspension into a standard circle scratched on a glass slide. Peterson (33) modified this method by using a round cover slip pressed into petrolatum coating on a glass slide. The fungicide was applied to this standard area. The amount applied was measured by the number of drops.

The method employed in these studies utilized a circle one square centimeter in area drawn on the slide with a wax pencil which was found to adhere very readily to the Pyralin surface. Ten circles can easily be drawn on a 1 × 3 inch slide. The area is covered with 0.1 ml. of fungicide suspension from a graduated pipette. Slight agitation of the pipette tip is necessary to spread the suspension over the entire area. The ordinary procedure is to make up the highest concentration to be used and to make a series of dilutions according to recommended ratios (1, 2). A 0.1-ml. sample of each dilution is pipetted into duplicate circles as soon as prepared and a sufficient quantity is retained for the next dilution, the remainder being discarded.

By dividing the percentage concentration in the fungicide suspension by 1000 a figure is obtained which expresses the amount of fungicide in micrograms per square centimeter. Multiplying this value by 10 will give a reading in parts per million assuming that all of the deposit exerts fungicidal action when re-wetted with the spore suspension.

Application of spore suspension. Correlated with the necessity for a uniform deposit is the problem of applying the spore suspension on a known area. The amount of deposit covered by the spore drop has been found to be an important variable by Horsfall et al. (12) and methods involving the spraying of the whole slide do not make provision for standardizing this area. Where the area is limited by a circle the amount of spore suspension has been standardized by applying a given number of drops (33) or a measured quantity (29, 30, 40).

In these studies the spore suspension was applied with a graduated pipette also. Exactly 0.1 ml. of suspension was placed on the dried fungicide so that the deposit was diluted to its original concentration. Thus only 2 ml. of spore suspension were required for each material being evaluated.

Incubation of spores. The recommended method (1) suggests the use of moist chambers inverted and sealed with water. Young (40) stacked Petri plate lids separated with glass plates and covered them with a bell jar. Culture dishes (150 or 200 mm. × 20 mm.) gave sufficiently high humidity for germination and were comparable to larger moist chambers. No loss in water could be detected even though they were not inverted and sealed with

TABLE 1.—*The effect of certain variable factors on percentage germination, LD50 values on a Bordeaux deposit, and coefficients of variation expressed in terms of statistical significance. Six replications on different days.*

Factor	Substances added	Sclerotinia fructicola	Venturia inaequalis	Phytophthora infestans	Alternaria oleracea
Juice concentration	Orange juice	**[a]	**		
Replicate experiments	Do	**	**	**	
24 vs. 48 hours	Do	**	**	**	
Replicate drops	Do	none	*[b]	**	
Juice lot	Do	**	**		
Germination surfaces	Do	none	none	none	
Tenacity coefficient of surfaces	Do	none	none		
Extract concentration	Lima bean	**	**	**	**
Citrate concentration	K & Na citrate		**	**	**
K vs. Na citrate	Do	none	**	*	**
Replicate experiments	Do	*	**	**	*
Sucrose vs. no sucrose	Do	none		*	
Sucrose concentration	Do	none		**	
Age of culture	Do			**	
Coefficient of variation	Bean, juice	20.7	14.1	13.4	16.7
Do	Dextrose	16.7	12.6	13.2	
Do		113.8			

a ** indicates significance at odds 99 : 1.
b * indicates significance at odds 19 : 1.
c Glass vs. coated glass vs. Pyralin.
d Actually 0.5 per cent sucrose.
e Difco lima bean extract—*P. infestans*; Orange juice—*S. fructicola*; *A. oleracea*.

water. Filter paper had no inhibiting effect on germination as compared to water only in the bottom of the dish.

The recommended method (1) specifies temperatures of 20° to 25° C. for spore germination. All spores were held at 18° C. for germination tests reported here unless otherwise indicated. As noted in table 1, 48 hours at this temperature gave a significantly different LD50 value from those held only for 24 hours. This effect of time has also been studied by Wellman and McCallan (38). In these studies the recommended (1) period of 20 to 24 hours was used at all times.

SPORE PRODUCTION

Sclerotinia fructicola and Alternaria oleracea.[2] The method of culturing and producing spores of these two fungi is fairly well standardized (1). Considerable variation was experienced in the yield of *S. fructicola*[3] on the recommended potato-dextrose agar and yields were not so high as those obtained by McCallan et al. (20). Sporulation was much higher on malt agar (Table 2) and more uniform (28). The 10 per cent malt gave the best yield.

Venturia inaequalis.[4] Reddick and Wallace (34) who first used *V. inaequalis* in laboratory slide tests, and several others (6, 24, 25, 37), obtained spores from leaves. Montgomery and Moore (30) produced the conidia on sterilized apple twigs. The same authors (29) also used a thin layer of malt agar to coat the inside of a test tube. Palmiter (31) and Keitt and Palmiter (15) described a method of producing conidia on a cheesecloth wick dipped in malt extract solution.

Since *Venturia inaequalis* does not grow extensively when transferred with a needle, it was necessary to use a method which would produce a uniform growth over the whole surface of the media in order to obtain maximum spore production per tube. This was accomplished by adding sterile water to a tube of the culture and scraping the surface with a transfer needle to make a spore suspension. This suspension was poured from one tube of media to the other. Sufficient numbers of spores lodge on the agar surface to give a good growth over the whole surface.

Relatively little attention has been given to factors influencing sporulation of *Venturia inaequalis* in culture. Montgomery and Moore (30) found 14 days to be optimum for production of conidia on twigs in culture tubes. Keitt and Palmiter (15) obtained good conidial yield in 10 days at 16° C. The number of conidia obtainable in water by rubbing the agar surface was highest at 21° and 9° C. (Fig. 1) indicating a double maximum curve. Only seven days were required at 21° C., while 25 were necessary at 9° C. Maximum yield was not reached at 6° C. within 35 days.

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[2] Cultures of *Sclerotinia fructicola* and *Alternaria oleracea* were obtained from Dr. McCallan, Boyce Thompson Institute, Yonkers, New York.

[3] All spores in this study were removed by rubbing in water with a rubber policeman as recommended (1).

[4] *Venturia inaequalis* was isolated from ascospores discharged from apple leaf onto malt agar.
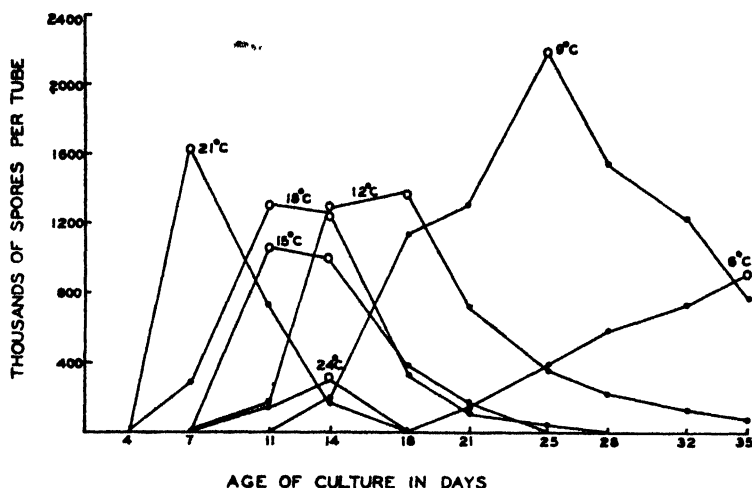
FIG. 1. *Venturia inaequalis.* Relation of age of culture and temperature to yield of conidia on 5 per cent malt agar. Three replicates of two tubes.

Conidial production was also influenced by malt concentration (Fig. 2). Ten per cent malt was best at 15° and 18° C.

Using three different lots of spores consecutively in transferring (three sowings on one tube) gave a significantly higher (approximately one-third) yield of spores than using one lot. Three tubes were grown at three different temperatures (12°, 15°, and 18° C.). Autoclaving the malt agar media longer than 20 minutes decreased the yield of conidia (Fig. 3). Steaming the malt showed no effect as compared with suspending it in hot water before tubing. Concentration of spores at time of transfer had little effect on conidial yield provided there were 200,000 or more per ml. Riboflavin (0.0125 to 0.1 per cent) had no effect on number of conidia per tube.
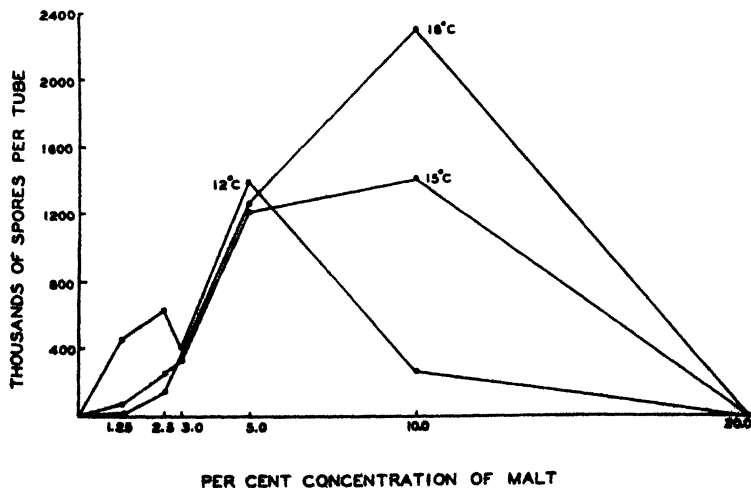
FIG. 2. *V. inaequalis.* Malt concentration and conidial production at different temperatures. Six replicates of two tubes at 14 days.

TABLE 2.—*Yield of conidia of Sclerotinia fructicola on different concentrations of malt, Difco malt, and potato-dextrose agar. Six replicates of two tubes at seven days*

Agar media	Percentage Malt	Thousands of spores per tube
Potato dextrose[a]	....	1431.3
Potato dextrose[b]	......	4516.5
Malt	1.25	2439.5
Do	2.50	3814.6
Do	5.00	4891.0
Do	10.00	6408.4
Do	20.00	3222.9
Malt (Difco)	1.00	1560.4

[a] 200 gm. potatoes and 20 gm. dextrose.
[b] 100 gm. potato ground in blender and all added to media without heating previous to autoclaving.

Phytophthora infestans.[5]   The fact that pure cultures are required for the slide germination technique precluded the use of the raw potato disc method developed by Crosier (4). Miller (28) found that addition of various materials, including vitamin C (32), to potato-dextrose agar did not give a sporangial yield equal to that of Lima bean agar. The latter was first used by Clinton (3) and was made by steaming 100 gm. of ground Lima beans in 1000 ml. of water and filtering through cheesecloth or a coarse sieve. This was added to 400 cc. of water in which 17 gm. of agar had been dissolved. The final volume was adjusted to 1000 cc., tubed, and autoclaved. Cultures have been carried for several years on this medium.
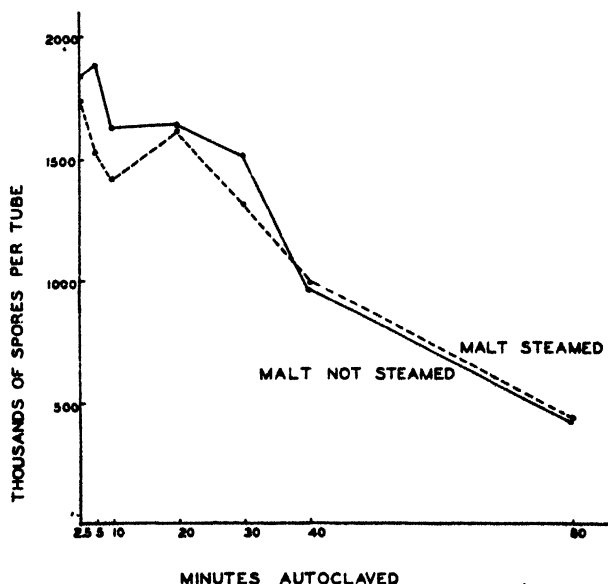
Fig. 3. *V. inaequalis.* Time of autoclaving 5 per cent malt agar and yield. Six replicates of two tubes in 10 days.

[5] *Phytophthora infestans* culture was obtained from a potato tuber from W. R. Mills of this station and is designated as the AAB₂ race.
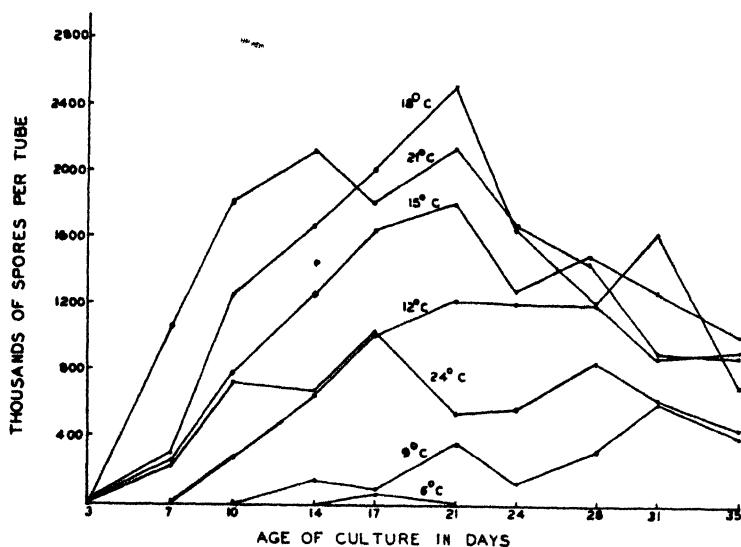
FIG. 4. *Phytophthora infestans.* Yield of sporangia and age of culture at different temperatures. Three replicates of two tubes.

Seventeen to twenty-one days were required for cultures to give highest yield at the optimum temperature of 18° C. (Fig. 4 and 5).   Clinton (3) also found growth best around 19° C.   Sniezko et al. (35) reported yields of sporangia on peanut hull grain media up to 2,000,000 per gm. dry weight
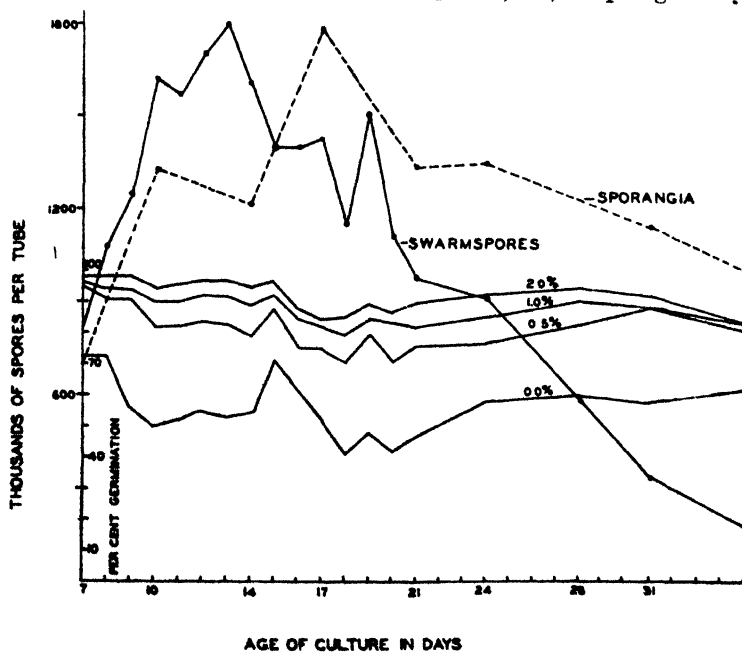
FIG. 5. *P. infestans.* Age of culture grown at 18° C. and yield of sporangia and swarmspores. Also relation of culture age to germination of swarmspores at different sucrose concentrations and 0.005 per cent K citrate. Six replicates of two tubes.
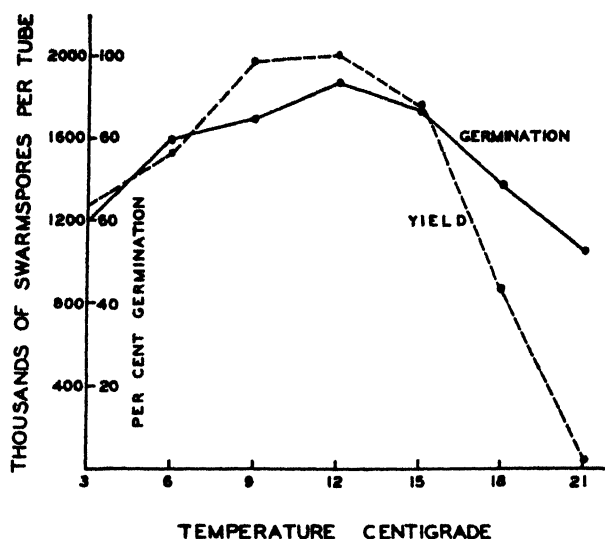
FIG. 6. *P. infestans.* Temperature at which swarmspores are formed, yield and germination. Six replicates of two tubes.

which is comparable to the 2,500,000 at 18° C. on the Lima bean agar (each tube contained approximately one gram dry weight).

Swarmspores form readily if the sporangial suspension is merely placed at a favorable temperature, as found by Melhus (26) and Crosier (5). The optimum temperature for germination of centrifuged sporangia was 12° C. (Fig. 6) which corresponds very closely to that found by these workers.

Crosier (5) obtained data which seemed to indicate that bentonite increased the germination of sporangia obtained from potato tubers. A statistically significant reduction in swarmspore yield was caused by the addition of bentonite to centrifuged suspensions of sporangia in glass-distilled, ordinary distilled,[6] and tap water (Table 3). No increase in sporangial

TABLE 3.—*Yield and germination of Phytophthora infestans swarmspores in different kinds of water with and without bentonite. Sporangia at 12° for three hours. Average of six replications*

Kind of water	Swarmspores per tube	Germination	
		No nutrients	K citrate + Sucrose[a]
	Thousands	*Per cent*	*Per cent*
Distilled	2,847	3.5	95.2
Do + Bentonite	1,845		
Tap	2,800	39.9	95.2
Do + Bentonite	2,448		
Glass distilled	3,418	64.9	93.3
Do + Bentonite	2,391		

[a] 0.005 per cent K citrate and 0.1 per cent sucrose.

[6] Ordinary distilled water was obtained from a metal still with a storage tank lined with tin.

germination resulted with vaseline (4, 36). Yield was highest in glass distilled water.

The time required for maximum swarmspore yield was found by Crosier (5) to be three hours at 12° C. McCallan and Wellman (19) found six hours were required at 10° C. In these studies maximum yield of swarmspores was secured in three hours in a 5-ml. suspension at 12° C. (Fig. 7). After this time the number that could be recovered decreased because of loss of motility and consequent settling to the bottom of the dish. These could not be dislodged by moderate agitation of the suspension. Amount and temperature of water used to make the sporangial suspension will no
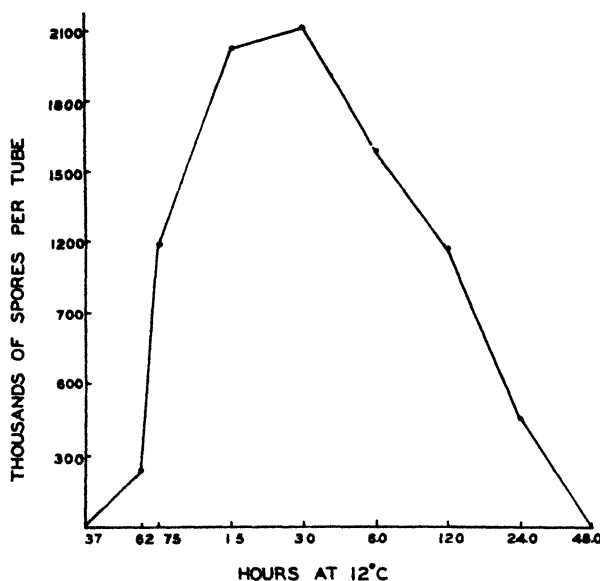
FIG. 7. *P. infestans.* Time at 12° C. and swarmspore yield of a 5-cc. suspension. Seven replicates of one tube.

doubt influence the time required. Three hours at 12° C. was used as a standard unless otherwise indicated.

Age of the original culture also influenced the formation of swarmspores (Fig. 5); cultures 10 to 14 days old gave the highest yield at 18° C. Ten days was used as a standard age. Agitation of the original suspension when removed from the culture tube increased the yield of swarmspores, a factor which must be due to the rapid settling rate of the large sporangia.

Depth of water in the germinating dish influenced swarmspore yield. Approximately one-third more swarmspores were obtained in 0.75 inch of water than in 2.5 inches. Five to ten ml. of water in a small (30 × 50 mm.) preparation dish has been satisfactory for each tube of *Phytophthora infestans.*

Factors that were varied but did not influence swarmspore formation were as follows: length of time water was in original culture tube; concentration of sporangia; temperature (6°–20° C.) of water used to remove

sporangia; length of time of centrifuging; number (0–3) of washings of sporangia by centrifuging; addition of sucrose.

SPORE GERMINATION AND DOSAGE RESPONSE

The conditions necessary for germination of fungus spores in water has received considerable attention (5, 6, 7, 8, 14, 26, 39). Goldsworthy and Green (10) found that coenzyme R would give good germination of *Sclerotinia fructicola*. McCallan and Wilcoxon (21) and Peterson (33) pointed out that varying amounts of media on which this organism was grown were removed in the process of making the spore suspension and were related to the varying percentage of germination. Peterson found an extract of Difco potato-dextrose agar would insure satisfactory germination. McCallan and Wilcoxon (21) eliminated this variable by centrifuging the spore suspension and adding a standard concentration of orange juice. The standardized procedure (1) recommends 0.1 per cent ultra-filtered orange juice for *S. fructicola* and *Alternaria oleracea*.

The importance of glass-distilled water was studied by Lin (16) and Marsh (23). With *Sclerotinia fructicola*[7] (Table 4), glass-distilled water gave significantly (99:1) better germination on check slides than ordinary distilled water with only Bacto-dextrose added. With *Phytophthora infestans* (Table 3) ordinary distilled water alone allowed almost no germination of swarmspores while with glass-distilled water over 50 per cent germinated, and with tap water germination was intermediate between these two.

Sclerotinia fructicola requires some additional substances after washing[8] the spores to give a range of 90 per cent or better, germination (Table 4) as recommended in the standardized method (1). Lin (16) found that 0.5 per cent purified dextrose permitted satisfactory germination. This was substantiated (Table 4), although no difference was found between purified (Difco-Bacto) and the technical grade of dextrose. $MgSO_4$, $CaSO_4$, and KH_2PO_4 were tried, but only $MgSO_4$ (0.1–20 millimols per liter) with 0.5 per cent sucrose gave the required increase in germination. Finally, potassium or sodium citrate and sucrose were tried and gave satisfactory germination (Table 4). Malic and citric acids also gave the same response with sucrose (Table 4). Washed spores of *Venturia inaequalis* and *Alternaria oleracea* also require added substances to give the required percentage germination and responded to the K and Na citrates and sucrose (Table 4). *A. oleraceae* was inhibited by malic and by citric acids with 0.5 per cent sucrose.

Phytophthora infestans swarmspores also failed to germinate satisfactorily when washed by centrifuging (Table 4). No response was obtained with orange juice (0.1 per cent) or with riboflavin (0.001 per cent). Malic acid and citric acid with 1.0 per cent sucrose inhibited germination.

[7] All germinations on slides were made at the recommended (1) spore concentration of 50,000 per ml.

[8] All spores were washed by centrifuging (1).

TABLE 4.—Percentage germination of washed spores on check slides. Average of six replications except malic and citric acids

Substances added	Distilled water	Sclerotinia fructicola	Venturia inaequalis	Alternaria oleracea	Phytophthora infestans
None	Ordinary	46.2	83.7	64.6	3.5
None	Glass	42.1	---	---	64.9
0.1 per cent Tech. Dextrose	Do	89.9	92.5	---	---
0.1 per cent Bacto-Dextrose	Do	87.7	93.3	---	---
Do	Ordinary	54.4	90.1	77.0	73.8
K citrate[a]	Do[b]	64.2	94.8	73.6	88.9
Sucrose[a]	Do[b]	80.8	95.2	96.8	95.8
K citrate + Sucrose[a]	Do[b]	99.0	96.3	97.8	42.8
Na citrate + Sucrose[a]	Do[b]	95.8	95.8	75.3	71.1
0.1 per cent Orange juice	Do	95.7			95.7
0.1 per cent Difco extract	Do				
0.001 per cent Malic acid[c]	Do[b]	91.0	94.8	0.0	9.0
0.001 per cent Citric acid[c]	Do[b]	97.8	92.3	3.1	0.0

[a] S. fructicola, V. inaequalis, A. oleracea—0.001 per cent K and Na citrate and 0.1 per cent K; P. infestans—0.005 per cent K citrate and 2.0 per cent sucrose.
[b] Glass distilled for P. infestans.
[c] S. fructicola, V. inaequalis, A. oleracea—0.5 per cent sucrose; P. infestans—1.0 per cent sucrose.

With Difco Lima bean agar extract (0.1 per cent) there was 95 per cent germination; and with 0.005 per cent potassium citrate and 2.0 per cent sucrose germination also was satisfactory (Table 4). Germination was related to concentration of sucrose (Fig. 5) with 2.0 per cent of the sugar bringing about the best response.

Age and temperature at which spores are grown influence the percentage germination (17, 20). McCallan (17) suggested that the optimum temperature for spore production of *Venturia inaequalis* was different from that for best spore germination. At 18° C., 90 per cent or more of the spores of *Venturia inaequalis* from cultures 7 to 14 days old germinated; at 15° C., as high a percentage germination occurred with spores from cultures 11 to 14 days old; and at 12° C., with spores from cultures 11 to 18 days old (Fig. 8). *Phytophthora infestans* (Fig. 5) swarmspores germinated best at 18° C. from cultures 7 to 15 days old.
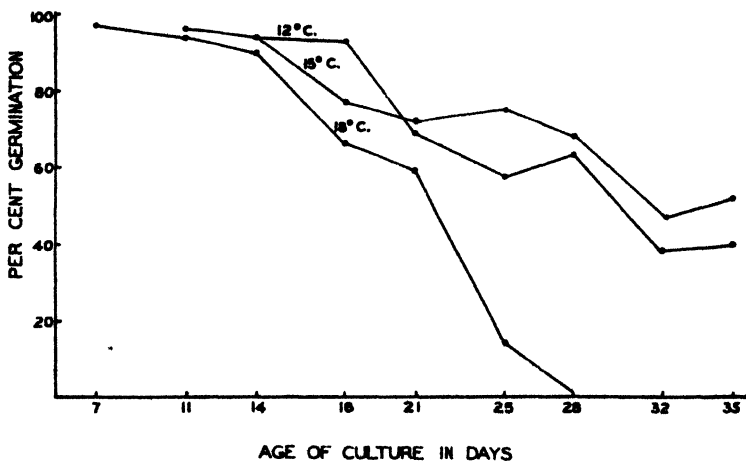
FIG. 8. *V. inaequalis.* Age of culture at different temperatures and conidial germination. Six replicates of one tube.

Temperatures at which swarmspores of *Phytophthora infestans* are formed have an effect on germination at 18° C. (Fig. 6). Those formed at 12° C. gave the highest percentage germination.

Henry and Smith (11) found that sufficient chromate remained on glassware cleaned with sulfuric acid-dichromate mixture to be toxic to microorganisms even after many rinsings with water. No evidence could be obtained for quantities remaining on glassware sufficient to inhibit germination of *Phytophthora infestans* swarmspores.

Other factors that did not influence germination of this organism were: centrifuging sporangia one to three times; holding sporangial suspension at 12° C. for 45 min. to six hours; concentration of swarmspores; diluting one to ten minutes after removal from 12° C.; temperature of water to dilute swarmspores, and type of water in which glassware was rinsed.

Miller (27) pointed out the linear relationship between increasing concentration of orange juice or citrate and higher LD50 values on a Bordeaux

mixture deposit. This emphasizes the importance of standardizing the concentration of these added substances. Also reported by the same author and noted also in table 1 was the variation in LD50 resulting from juice from different lots of oranges which led to the use of the pure chemicals, sodium and potassium citrate. Dextrose alone will insure good germination of *Sclerotinia fructicola,* but the LD50 values on Bordeaux mixture deposits not only failed to come within the range obtained with the standardized procedure using orange juice but also gave a much higher coefficient of variation (Table 1) as a result of a more gradual slope (13).

The role of nutrients or so-called "stimulants" has been the subject of considerable discussion. Lin (16) and Marsh (23) suggested that an increased germination with plant extracts was due to the neutralization of toxic materials in ordinary distilled water by salts that inactivate heavy metals and by proteins that would be contained in these decoctions. The effectiveness of pure citric acid with *Sclerotinia fructicola* would seem to exclude this theory. McCallan (correspondence, 1947) did use glass-distilled and not the ordinary distilled water, as suggested by Marsh, and thus the effect with orange juice could not be explained as a neutralization of toxic ions in ordinary distilled water.

Lin (16) reported that the LD90 of copper sulphate for spores of *Sclerotinia fructicola* was of the order of 0.3 p.p.m. using dextrose only. This agrees with a value of 0.42 p.p.m. on dried Bordeaux deposits found in the experiments using only dextrose with this organism. Where the citrates or orange juice was used, values, of course, were much higher.

STANDARDIZED PROCEDURES DEVELOPED IN THIS STUDY

The fungicide suspension (0.1 ml.) is placed in circles one square centimeter in area drawn with a wax pencil on Pyralin slides. In this area 0.1 ml. spore suspension is placed on the dried deposit and the spores are incubated in a large culture dish.

Sclerotinia fructicola is grown on 10 per cent malt agar at 18°–20° C. *Venturia inaequalis* is grown on 10 per cent malt for 10–12 days at the same temperature and is transferred by a mass sowing of a sterile water suspension of conidia. *Phytophthora infestans* is grown on Lima bean agar for 10 to 14 days. *P. infestans* swarmspores used for germination are secured by placing centrifuged sporangia at 12° C. for three hours in glass-distilled water less than one-half inch deep.

Potassium citrate (0.001 per cent) and sucrose (0.1 per cent) are used in place of orange juice for germination of centrifuged spore suspensions of all organisms except *Phytophthora infestans* which requires these substances at 0.005 per cent and 2 per cent respectively.

SUMMARY

Standardized procedures were developed for the use of *Venturia inaequalis* and *Phytophthora infestans* in slide germination evaluations of

fungicides.   Variations in the methods for *Sclerotinia fructicola* and *Alternaria oleraceae* were devised.

Venturia inaequalis produced highest conidial yields at 9° C. in 25 days and at 21° C. in 7 days.   The number of conidia that could be removed with water decreased markedly with age in *V. inaequalis*.   Swarmspore yield in *Phytophthora infestans* also decreased with age of culture.

When used as a nutrient, orange juice from different lots of oranges gave variable LD50 values.   Dextrose and glass-distilled water with *Sclerotinia fructicola* gave very flat dosage response curves with consequent lower and more variable LD50 values.

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OCCURRENCE OF THE BOTRYTIS DISEASE OF GLADIOLUS IN THE UNITED STATES IN RELATION TO TEMPERATURE AND HUMIDITY

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The Botrytis disease of gladiolus as described by Hawker (7) and Moore (14) in England, Timmermans (15) in Holland, Drayton (6) in Canada, and Wade (18) in New South Wales has two distinct phases: corm rot in storage, and a leaf and flower spot and collar rot in the field. This same disease occurs in the United States where it frequently becomes a serious problem in the commercial culture of gladiolus in Florida, California, Oregon, and Washington. Until recently, little attention has been given this disease in the United States although Drayton reported its interception in Canada as early as 1927 (6) on some corms grown in Oregon. According to McWhorter (12), a strain of *Botrytis cinerea* Pers. attacked maturing gladiolus foliage near Portland, Oregon, in 1939. Recently he has written us that "soft rot, due to *Botrytis cinerea* and possibly other species, has been the most destructive gladiolus disease in Oregon. Total loss from this disease has exceeded in one year the losses from all other diseases in five years." Dimock (4) reported an epiphytotic of *Botrytis* on gladiolus foliage in Florida in 1940, although he doubted that a specific gladiolus strain of *Botrytis* was involved. Tisdale (17) questioned Dimock's claim that *Botrytis* was the causal agent. In the same year, Weiss (19) reported a Botrytis dry rot of gladiolus corms on Long Island, and Dodge and Laskaris (5) reported it from the same area the following year. Magie (13) recently discussed the occurrence of Botrytis, Stemphylium, and Curvularia leaf spots as they occur in Florida, and we have discussed the prevalence of the Botrytis disease in the United States (11). The Botrytis disease is now the limiting factor in the culture of gladiolus in California (2).

The present paper presents data concerning the effects of temperature and humidity on the development of the disease and correlates this information with the known occurrence of the disease in the United States.

THE ORGANISM

Klebahn (8) described *Botrytis gladioli* Kleb. in 1930, and Timmermans (16) described *B. gladiolorum* Timmermans in 1942. *Botrytis gladioli* differs from *B. gladiolorum* mainly in the shape of the conidia

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which are long and narrow, averaging 10.4 μ × 4.7 μ, whereas conidia of the latter species are ovoid to subglobose and average 15 μ × 10 μ. Conidia of the *Botrytis* isolated by Moore (14), by Hawker (7), by Dodge and Laskaris (5), and by us correspond with those of *B. gladiolorum* (conidia from our isolates average 14.7 μ × 9.8 μ). Wade (17) called the *Botrytis* he studied *B. gladioli*, but presumably it was *B. gladiolorum* since his spore measurements and other morphological evidence agree with Timmermans' organism rather than with Klebahn's. Miss Hawker (7) considered her isolates to be strains of *B. cinerea* but the spore measurements she gives agree with Timmermans' *B. gladiolorum*. Dennis and Wakefield (3) described the perfect stage of a *Botrytis* isolated from a gladiolus corm by W. Buddin as *Sclerotinia Draytoni* Dennis and Wakefield. They described the conidia as narrowly elliptical and 8–16 μ × 5–7.5 μ. These spore measurements agree with those of *B. gladioli*. Drayton obtained single ascospore cultures from Buddin and in a personal communication to us states, "After making a number of measurements, Dr. Groves and I are of the opinion that *Botrytis gladiolorum* is the conidial stage of *Sclerotinia (Botryotinia) Draytoni*, and that this is the fungus responsible for the early-storage decay." Parallel inoculations of Picardy gladiolus corms with one of Buddin's isolates (163-K) obtained by us from Drayton and with one of our own isolates (213-D) have given similar infection (Table 2). *Botrytis gladioli* is poorly described and no mention is made of its effect on the plant. Timmermans points out that Klebahn mounted his spores in glycerine and his measurements might have been greater had the spores been mounted in water. MacLean (10) has reported gladiolus foliage infection with *B. elliptica* (Berk.) Cke. This infection produces foliage symptoms very much like those produced by *B. gladiolorum*. We consider the *Botrytis* used in our tests to be *B. gladiolorum*.

METHODS AND RESULTS

Foliage Infection

At the Plant Industry Station, Beltsville, Maryland, gladiolus plants were inoculated artificially and kept at different temperatures. For the first inoculation test, Picardy corms were planted, one per pot, in soil in the greenhouse October 2, 1946, and 50 plants were selected for uniformity October 31. Forty of these were inoculated by atomizing a spore and mycelial suspension of *Botrytis* on the foliage with a deVilbiss atomizer. This *Botrytis* was a single spore isolate (213-C) made May 1, 1946, from infected gladiolus grown at Redondo Beach, California. Of 32 single spore isolates made at this time, one produced conidia abundantly, four produced both sclerotia and mycelium, and the rest produced neither sclerotia nor conidia. Eight of the inoculated plants and two of the non-inoculated plants were then placed in each of five temperature chambers (35°, 40°, 55°, 65°, and 75° F.) and the humidity was maintained at, or

near, saturation by surrounding the plants with wet cheesecloth. Two inoculated plants were removed from each temperature to a greenhouse maintained at 70° F. daytime and 60° F. night, after each of the following intervals: 24, 72, 120, and 168 hours. The noninoculated plants were removed after 168 hours. In another test, pairs of Picardy, Early Rose, and Pacifica varieties of gladiolus plants were inoculated in a similar manner with isolate 213-D and removed from the five temperatures after 24, 48, 96, 144, and 240 hours. Numbers of Botrytis lesions on Picardy are given in table 1. Similar data were obtained from the Early Rose and Pacifica varieties. These lesions (Fig. 1) were typical of those seen in the field. There was slight infection at 75° F.; infection progressed rapidly at 65° F., less so at 55°, 45°, or 40° F., and there was very little at

TABLE 1.—*Botrytis infection of the foliage of the Picardy variety of gladiolus as affected by time and temperature*

Isolate of Botrytis used	Incubation time[a]	Temperature in degrees F.				
		35	45[b]	55[c]	65[c]	75
	Hours	*Number of lesions on two plants*				
213-C	24	2	2	43	34	15
	72	3	77	131	405	3
	120	43	844	429	[d]	31
	168	97	685	221	[d]	79
213-D	24	0[e]	1	5	26	9
	48	9	0[e]	16	176	38
	96	30	50	23	804	25
	144	48	75	399	147[e]	16
	240	63	640	195[c]	280[e]	40

a After inoculation the plants were kept in a saturated atmosphere at each of the temperatures for the designated periods of time, then moved to a greenhouse maintained at 65° to 70° F. where the humidity was moderate.

b Foliage inoculated with isolate 213-C was incubated at 40° F. instead of 45°.

c The lesions on plants incubated at 55° and 65° F. were larger than those on plants incubated at 35° or 45° F., and these tended to coalesce without the typical red necrosis, thus reducing lesion counts.

d The foliage was nearly dead after 72 hours and accurate lesion counts could not be made.

e Number of lesions on one plant instead of two plants.

35° F. Infection was most rapid at 65° F. and the lesions were largest at this temperature. At 35° F. the lesions were small, pin-point necrotic spots, about 1 mm. in diameter. At 40° F. or 45° F. the pin-point lesions tended to coalesce and become larger (3 mm.) after 6 to 10 days. The lesions on the plants at 55° F. were larger than at lower temperatures and tended to coalesce without the typical red necrosis. The entire leaf tips were killed after 120 to 168 hours, which resulted in fewer lesions being counted. After 6 to 10 days, many pin-point lesions developed and these were thought to be due to secondary infection. Twenty-five lesions developed on the noninoculated Picardy plants held at 65° F. for 168 hours. They were probably due to infection from spores blown from the non-

inoculated plants kept in the same chamber. Thus, the optimum temperature for the leaf-spotting phase of the disease is about 55° to 65° F. with slightly less infection at 40° to 45° F. Thirty-five degrees F. is below the point of rapid infection and 75° F. is above it. Consequently, the leaf spot and the collar rot phases of this disease would be expected to be most severe in areas where temperatures are low and humidity is high during the growing season. The disease could also be severe late in the season or during short periods of cool, wet weather. Both Wade (18) and Timmermans (15, 16) obtained optimum growth of the fungus in culture between 68° and 71.6° F., and Wade points out that this is in agreement with

FIG. 1. Picardy gladiolus inoculated with *Botrytis gladiolorum* and incubated in a saturated atmosphere at the following temperatures, left to right, 35°, 40°, 55°, 65°, and 75° F. for 168 hours before removal to a greenhouse maintained at 65° to 70° F. Photographed by Pryor 15 days after inoculation.

field observations that the disease becomes most severe in Australia late in the season when temperatures are low and humidities are high.

Corm Infection

Picardy gladiolus corms were inoculated at Beltsville by cutting them in three places with a scalpel and dipping them in a spore and mycelial suspension of *Botrytis*. These corms were stored in moist chambers at 35°, 45°, 55°, 65°, and 75° F. In one test, 100 corms were dug March 13, 1947, and 20 corms were placed at each of the five temperatures. On March 14, ten corms from each temperature chamber were wounded, inoculated with isolate 213-D, and placed in moist chambers in each of their respective temperatures. The remaining ten corms from each temperature

were wounded similarly and placed in another set of moist chambers as
controls.   These corms were removed May 6 and examined for rot.   They
were photographed May 14 (Fig. 2, B)   There was no infection apparent
at 55°, 65°, or 75° F , slight infection appeared around the cuts at 45° F.;
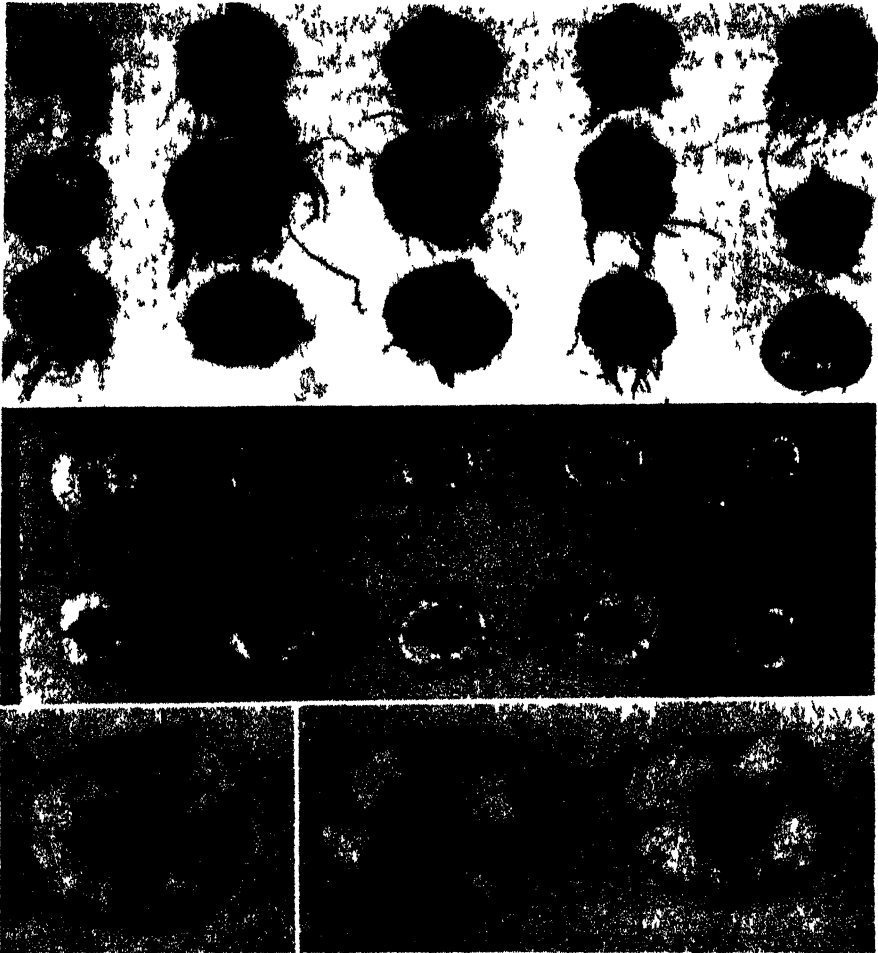
FIG 2   Picardy corms inoculated with Isolate 213D of *Botrytis gladiolorum* and
incubated (A and B), left to right, at 35°, 45°, 55°, 65°, and 75° F , and (C) at 35°
left, and 45° F. center and right.   Note the lighter color of the rot at 35° and the
absence of rot at 65° and 75° F.   A, Corms inoculated October 13, 1947, and photo
graphed by Pryor 67 days after inoculation   B, corms inoculated March 13, 1947, and
photographed 62 days after inoculation   The husks were removed before photographing.
C, cross sections of corms incubated at 35° left, and 45° F , center and right, for 62
days   Photograph by Mead

and infection was greatest with abundant mycelial growth and sporulation
at 35° F.   In a second test, corms were dug October 13, 1947, wounded by
cutting each corm with a scalpel in three places, and inoculated two hours
later by dipping them in a spore and mycelial suspension of *Botrytis*.

Two isolates were used, the 213-D isolate from California and the 163-K isolate from Buddin, 45 corms being inoculated with each isolate. Nine corms inoculated with each isolate were placed in moist chambers and stored at each of the five temperatures. Records were obtained December 23, and these data are given in table 2. Corms were rotted by *Fusarium* at the three highest temperatures and by *Botrytis* at the two lowest temperatures, although there was no rot among corms inoculated with isolate 213-D at 45° F. Mycelium of both isolates grew over the husks of the corms at the three lowest temperatures and sporulated abundantly. Sclerotia formed on corms inoculated with isolate 213-D (Fig. 3) at the three lowest temperatures and on corms inoculated with isolate 163-K stored at

TABLE 2.—*Infection of Picardy gladiolus corms with two isolates of Botrytis gladiolorum. Corms incubated at different temperatures for 67 days after inoculation*

Temperature (° F.)	No. corms with Botrytis fruiting[a]	No. corms with sclerotia	No. corms having rot	Organism isolated
		Isolate 213D		
35	9	7[b]	5	Botrytis
45	9	9	0	do
55	1	1	7	Fusarium
65	0	0	9	do
75	0	0	9	do
		Isolate 163K		
35	9	9[c]	3	Botrytis
45	8	0	1	do and Fusarium
55	3	0	3	Fusarium
65	0	0	8	do
75	0	0	9	do

a Nine corms were inoculated with each organism at each temperature.
b These sclerotia were immature when examined.
c These sclerotia were slightly smaller than those on corms inoculated with Isolate 213D.

35° F. These latter sclerotia were slightly smaller than those from isolate 213-D.

The appearance of those corms inoculated and held at 35° F. was much different from those held at higher temperatures (Fig. 2). Corms held at 45° F. or higher had darkened husks and the rotted areas were very dark, whereas the husks and rotted areas of those held at 35° F. were lighter in color and the rotted areas were not so well defined. This was true of corms inoculated March 14, 1947, and also of those inoculated October 13, 1947. The reason for these differences in color is not known. Hawker (7) points out that, although Moore (14) described three forms of rotting of gladiolus corms associated with species of *Botrytis*, his three forms of rotting may be merely expressions of the same disease as affected by temperature.

In another test in which mature corms were dug from pots in the greenhouse at Beltsville on July 21 and inoculated August 8, no *Botrytis* infection was present after 66 days at any temperature, although some Fusarium rot was present at 55°, 65°, and 75° F. Drayton (6) observed that infection of the corms and spread from corm to corm take place more rapidly at 38° and 45° F. than at higher temperature ranges. Furthermore, in one experiment he was unable to infect cured corms with *Botrytis*, whereas freshly dug corms rotted rapidly following inoculation. Hawker (7) and and Timmermans (15) report that very little corm rot occurs at temperatures above 68° F. even though the optimum temperature for growth of the *Botrytis* is about 70° F. When corm infection is arrested, the lesion

FIG 3. Mycelium and sclerotia of *Botrytis gladiolorum* on husks of Picardy corm inoculated with Isolate 213D and incubated at 45° F. for 67 days. Photograph by Pryor.

is surrounded by a layer of suberized cells which walls off the disease (6, 9, 18). Lauritzen and Wright (9) compared the results of Artschwager and Starrett's (1) studies of temperature and humidity on suberization and wound periderm formation in gladiolus with their results on the effect of temperature and humidity on corm rot in gladiolus (stated to be due mainly to *Penicillium*). They found that the temperatures or humidities favorable for rapid suberization and periderm formation prevented or inhibited infection. Thus, at temperatures above 71.5° F. suberization took place rapidly and infection was prevented, whereas at low temperatures suberization was much delayed and infection occurred. Corms stored at 40° F. for 79 days had very little wound periderm and at 59.5° F. wound periderm did not form until after 22 days. Suberization also

took place much more slowly at low humidities. The explanation for the low temperature requirements for *Botrytis* infection of gladiolus corms becomes clear in the light of this information. Undoubtedly, if it were not for rapid suberization and wound cork formation at higher temperatures, optimum temperature requirements for corm infection would more nearly parallel those for foliage infection.

Timmermans (15) concluded that rot was much more prevalent on corms dug late in the season than on those dug early. Wade (18) dug corms early, midseason, and late, and the percentage of Botrytis rot was 0.5, 27.1, and 21.7, respectively. Similar results were obtained by Hawker (7). In an experiment in which Picardy corms were dug at two-week intervals beginning September 24, 1946, at Puyallup, Washington, Botrytis

TABLE 3.—*Botrytis rot in Picardy gladiolus corms at Puyallup, Washington, in 1946, as affected by the digging date*

Digging date	Average daily rainfall between digging dates	Mean temperature[a]	Total corms harvested[b]	Corms with Botrytis rot	
	Inches	° *F.*	*Number*	*Number*	*Per cent*
Sept. 24		58.4	1275	31	2.43
	.109				
Oct. 8			1355	39	2.88
	.129	47.5			
Oct. 22			1360	61	4.48
	.053				
Nov. 5			1380	64	4.64
	.222	40.4			
Nov. 26			1352	197	14.57
	.340				
Dec. 16		39.8	1333	368	27.61

[a] These are mean temperatures for the months indicated.
[b] Data are based on the combined totals of two planting dates (April 11 and May 27).

rot was least in the early diggings and greatest in the corms dug last (Table 3). There was a progressive increase in rainfall and decrease in temperature during this time, so that conditions were favorable for development of Botrytis rot and poor for curing corms.

DISCUSSION

If we correlate the local climatic conditions under which gladioli are grown with the conditions that appear to be necessary for *Botrytis* infection, the reason for the prevalence of the disease in certain areas in the United States becomes evident. Corm rot is not a problem in Florida although leaf spotting becomes severe sporadically. In the commercial gladiolus areas of upstate New York and of Illinois, Indiana, and Michigan, *Botrytis* has not been reported on gladiolus. However, it has been reported as causing a corm rot of gladiolus grown on Long Island (2, 10). Dimock reported to us that Botrytis leaf spot occurred late in the fall of

1947 and that Botrytis corm rot was prevalent this past winter (1947–48) in upstate New York. There are no previous reports of the occurrence of this disease in upstate New York. Both the corm rot phase and the leaf spot phase of the disease are severe in Oregon, Washington, and California.

Gladioli are grown in upstate New York, Michigan, Illinois, Indiana, Washington, and Oregon during the summer months only, whereas in the coastal areas of Southern California they are grown commercially as an early spring crop flowering in April and May. In Florida gladioli are grown as a winter crop with the peak of flower production occurring in February and March. Florida gladioli are subject to "northers" during January, February, and March. These usually last for 3 or 4 days and are accompanied by cold, wet weather. Fogs in Florida are confined almost exclusively to the early mornings during the winter. It is during these times that *Botrytis* develops rapidly on the foliage and flowers, resulting in a big decrease in flower quality. Corm rot is not usually a problem in Florida, however, since the average temperature, even in January, in the gladiolus-producing areas is 60° to 65° F., which is sufficiently high to cure the corms rapidly and to wall off infection by suberization before the corms are placed in commercial storage at 40° F. Thus, temperature limits *Botrytis* in Florida to occasional epiphytotics of foliage spotting.

Temperatures during the growing season in the gladiolus areas of upstate New York, Michigan, and Illinois are generally too high to permit epiphytotics of the disease. The average July temperatures in these areas are 70° to 75° F. and these temperatures are accompanied by moderate humidities (average 60 to 80 per cent). Thus, except for wet periods early or late in the growing season, *Botrytis* should not generally be a factor in these areas.

In the coastal areas of California cool weather occurs during early spring when gladioli are grown there with temperatures averaging 54.6° to 62.2° F. from January through May at Los Angeles. This coolness, together with occasional periods of rain or heavy fog plus the general practice of using overhead irrigation, provides ideal weather conditions for the foliage phase of the disease. Gladiolus cannot be grown in this area for winter flowering primarily because of *Botrytis*. Corms are commonly held in cold storage in California. If they are dug when the weather is cool and not cured sufficiently before being placed in storage and if plenty of inoculum is available, the stage is set for an abundance of corm rot. This often occurs.

In western Washington the Botrytis blight of leaves and flowers does not generally become severe until the early fall rains and fogs begin. It is usually negligible during July when temperatures average 64° F. at Seattle, precipitation is only 0.6 of an inch, and the relative humidity is 85 per cent at 8:00 A.M. and 52 per cent at 8:00 P.M. August conditions

are much the same, but in September the average temperature is 59°, precipitation is 1.7, and relative humidity is 89 per cent at 8:00 A.M. and 62 at 8:00 P.M. The average number of hours of sunshine for July and September are 312 and 185, respectively. This average drops to 113 in October and 60 in November with an increase in relative humidity and precipitation, and a decrease in temperature. Total rainfall in June, July, and August totals 2.6 inches as compared to 9.5 inches for September, October, and November. The conditions therefore become progressively more favorable for development of the disease. The progressive increase in foliage and flower infection is also reflected in increased corm infection.

In the past many western Washington growers have carried bulbs in common storage, but as a result of the rapid increase in severity of the *Botrytis* corm rot, most of the commercial growers have obtained equipment and designed storage facilities so that corms can be dried rapidly after digging. This is supplemented by culling, treating before planting, cutting and burning of unsold spikes, and in some cases by spraying plants with Bordeaux.

In contrast to the common occurrence of Botrytis corm rot in western Washington under its cool, moist conditions is the relative absence of this disease in eastern Washington, under drier, warmer summer and fall conditions. Most of the limited commercial acreage in eastern Washington is concentrated around Sunnyside, in the Yakima Valley. July temperatures average 71.7° F., with a precipitation of 0.20 inches. September temperatures average 61.0° F., with 0.54 inches of rainfall. Relative humidity data are not available for Sunnyside but at Yakima they are 52 and 20 per cent (8:00 A.M. and 8:00 P.M., respectively) in July, and 67 and 33 per cent in September. An examination of stocks of three of the largest growers in this area in the winter of 1947 failed to disclose a single case of Botrytis corm rot, even though the corms had been held in barn or cellar storage under moist conditions since digging.

It would appear from the foregoing that the occurrence and prevalence of both phases of the Botrytis disease of gladiolus could be predicted with the aid of weather records. If, for instance, a new area were being considered for gladiolus production and it was found on consulting the weather records that the temperatures in this area averaged between 55° and 65° F. during a four-month growing period, and if these temperatures were accompanied by abundant rainfall or heavy fogs, gladiolus production would most likely be very hazardous because of *Botrytis*. If, on the other hand, these same temperatures prevailed but were not accompanied by frequent rain or foggy weather, *Botrytis* would not be expected to be hazardous to gladiolus production in the area. Similarly, if temperatures averaging 70° F. or more prevailed later in the season, gladiolus could then be grown with safety.

SUMMARY

Infection of the foliage of three varieties of gladiolus with *Botrytis gladiolorum* Timmermans under controlled temperature conditions was most severe at 55° and 65° F. Thirty-five degrees was below the point of rapid infection and 75° F. was above it.

The optimum temperature for infection of freshly harvested corms was 35° F. Some infection also took place at 45° F., but none at 55° F. or higher. Infection did not occur on corms that had been allowed to cure for 18 days. Lack of infection of cured corms and of those inoculated and incubated at high temperatures was assumed to be due to the rapid suberization and wound periderm formation that took place at the higher temperatures.

Corms inoculated and incubated at 45° F. or higher had darkened husks and the rotted areas were very dark, whereas the husks and rotted areas of those held at 35° F. were lighter in color and the rotted areas were not so well defined.

Similar corm infection, sporulation, and sclerotial formation occurred from inoculations made with a single spore isolate of *Botrytis* from gladiolus from California and with a single ascospore isolate of *Sclerotinia Draytoni* Dennis and Wakefield.

The percentage of Botrytis rot in Picardy corms was least in early diggings and greatest in the corms dug last, when corms were dug at two-week intervals at Puyallup, Washington.

The occurrence of the disease in the United States is discussed in the light of the data presented and it is concluded that the regional prevalence of both the foliage phase and the corm rot phase of the disease in a given area can be predicted with the aid of weather records.

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EFFICACY OF SOIL FUMIGANTS AS AFFECTED BY SOIL TEMPERATURE AND MOISTURE

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INTRODUCTION

There have been numerous references cautioning against the use of soil fumigants when temperatures are low. Godfrey and Young (3) mention that soil treatments are most effective at temperatures above 70° F., and they state, "For best results with chloropicrin, the temperature of the soil at a depth of 6 inches should be warmer than 65°. Rapidity of penetration and killing increases with rises in temperature. . . . Most of the fumigants are inefficient below 45° F." Newhall, Chupp, and Guterman (6) state that a temperature above 65° is "ideal for fumigation." There are numerous other references alluding to the fact that low soil temperatures decrease the efficacy of soil fumigants. However, a search of the literature yields very little experimental evidence in support of this contention. In fact, Parris (9) observed that D-D was effective when used in soil having a temperature of 38° to 40°. Stark (11) found a significant reduction in the efficacy of chloropicrin when the soil temperature at the time of treatment was reduced from 85° to 73°. Newhall and Lear (7), on the other hand, reported that methyl bromide treatment of soil in sealed drums was just as effective at 48° as at 60°. They also reported good results when Dowfume G was used to treat soil at 52°.

Hagan (4) and Hannesson (5) have both studied extensively the movement of carbon disulfide in soils. They found that the soil moisture content very markedly affects the permeability of the carbon disulfide. Hagan states that moisture affects permeability more than any other factor studied and Hannesson reports that the movement of carbon disulfide is 6 to 10 times greater through air-dry soil than through wet soil. According to Stark (11), high moisture markedly reduces the effectiveness of chloropicrin, although it is commonly recommended that soils be moderately moist for fumigation (1, 3, 6).

To gain information on the effect of temperature and moisture on the efficacy of soil fumigants, tests were made in the greenhouses at the Plant Industry Station, Beltsville, Maryland, in 1946 and the results of these tests are presented here.

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METHODS

The tests were carried out in soil temperature tanks of the Wisconsin type in a greenhouse maintained at 70° to 75° F., although late in the spring air temperatures frequently rose to 90°. There were 6 tanks and the following soil temperatures were maintained: 45° to 50°, 55°, 65°, 75°, 85°, and 95°. No cooling system was used in the tanks, however, and with warmer weather the 45° to 50° tank usually ran at 54° to 55° or higher. There was some variation in the temperatures in the other tanks also (see tables 1, 3, and 4). In each tank there were 4 rectangular cans, each 10 inches wide by 18 inches long by 9 inches deep. Forty-six and a half pounds of an air-dry soil mixture consisting of 2 parts of a heavy composted clay loam and 3 parts of sand were put in each can. The moisture level of 2 of the cans in each tank was maintained at 7 to 10 per cent, and the moisture level in the other 2 cans was kept at 17 to 20 per cent. The cans were weighed daily and water was added when needed. Thus, half of each test was carried out in dry soil and half in wet soil.

The three organisms used in the tests were: the aster-wilt Fusarium, *Fusarium oxysporum* f. *callistephi* (Beach) Snyder & Hansen; *Sclerotium rolfsii* Sacc.; and the root-knot nematode, *Heterodera marioni* (Cornu) Goodey.

For each test the aster-wilt Fusarium was grown on sterilized oats in quart jars for approximately 30 days and then ground in an Enterprise meat grinder. A large teaspoonful of this mixture was put in each of 120 cheesecloth bags. *Sclerotium rolfsii* was grown on a modified Tochinai agar in Petri dishes until sclerotia were well formed at which time they were counted into 120 cheesecloth bags, 50 sclerotia per bag. Root-knot-nematode galls on tomato roots were ground in a food chopper and mixed with moist sand and about 2 teaspoonfuls of this mixture placed in each of 120 cheesecloth bags. While this inoculum contained undecomposed root material, the galls and roots were cut into small pieces.

Five bags each of *Fusarium oxysporum* f. *callistephi* and of *Sclerotium rolfsii* were buried in one end of each can and 5 bags containing *Heterodera marioni* were buried in a similar position at the opposite end. Two and a half ml. of the fumigant was placed 3 inches deep in the center of each can and covered with soil. This point of injection was about 7¼ inches from the buried bags. No water seal or other seal was used. One bag of each of the 3 organisms was removed at the following intervals after the application of the fumigant: 1, 3, 6, 9, and 13 or 14 days. In some of the tests, bags containing the organisms were buried in the centers of the cans 1, 3, and 6 days after the application of the fumigant to give information on the retention of the fumigant in the soil. These bags were all removed 14 days after the application of the fumigant.

After the organisms were removed from the soil they were tested for viability. The oat mixture containing the Fusarium was placed in 250-ml.

beakers, water added, and the mixture stirred. Roots of 2 young aster seedlings (about 1 month old) of the susceptible variety Super Giant El Monte were dipped in the suspension and planted in pots of steamed soil in the greenhouse. Death of the aster plants indicated viability of the organism (Fig 1). The sclerotia of *Sclerotium rolfsii* were planted in Petri dishes on moist Mosinee paper toweling. Percentage of germination was the criterion of viability (Fig. 2). Bags containing the root-knot galls were mixed with steamed soil in pots in the greenhouse and squash seeds were planted in the soil. After 6 weeks the plants were removed from the soil, the roots ex-
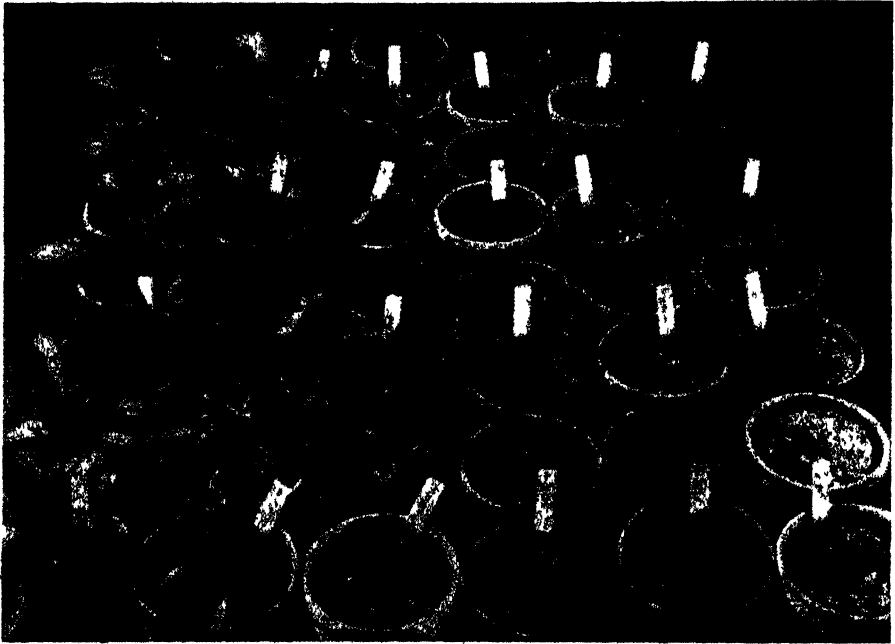
Fig. 1. Aster seedlings one month after inoculation with samples of *Fusarium oxysporum f. callistephi* that had been exposed for one day to Larvacide in the soil at the following temperatures, left to right: 98°, 81°, 70–75°, 57–59°, 46–48°, and 45–56° F. The first four plants in each row were inoculated with *F. oxysporum f. callistephi* that had been exposed to Larvacide in dry soil and the other four with *F. oxysporum f. callistephi* that had been exposed in wet soil.

amined, and given a relative root-knot index (10). This rating served as a criterion of nematode viability.

The soil fumigants used in these tests were: D-D (a mixture of dichloropropene and dichloropropane), Larvacide (chloropicrin), Dowfume G (10 per cent methyl bromide by volume in a mixture of 3 parts ethylene dichloride and 1 part carbon tetrachloride), and Dowfume W-15 (15 per cent ethylene dibromide by volume in a naphtha thinner).

<div align="center">RESULTS</div>

Larvacide. The effect of temperature on the efficacy of Larvacide is given in table 1. In this experiment the soil in but 2 of the 4 cans in each

temperature tank was treated with Larvacide; the soil in the other 2 was not treated   Bags of inoculum of the organisms were buried in the soil in each of the cans   Thus, information was obtained on the effect of time and temperature on the inoculum in the untreated soil as compared with that exposed to Larvacide

Larvacide was not effective against the nematodes except at 98° F   Apparently the bags of root-knot inoculum were just outside the effective killing range of the dosage of chloropicrin used, although the effective killing
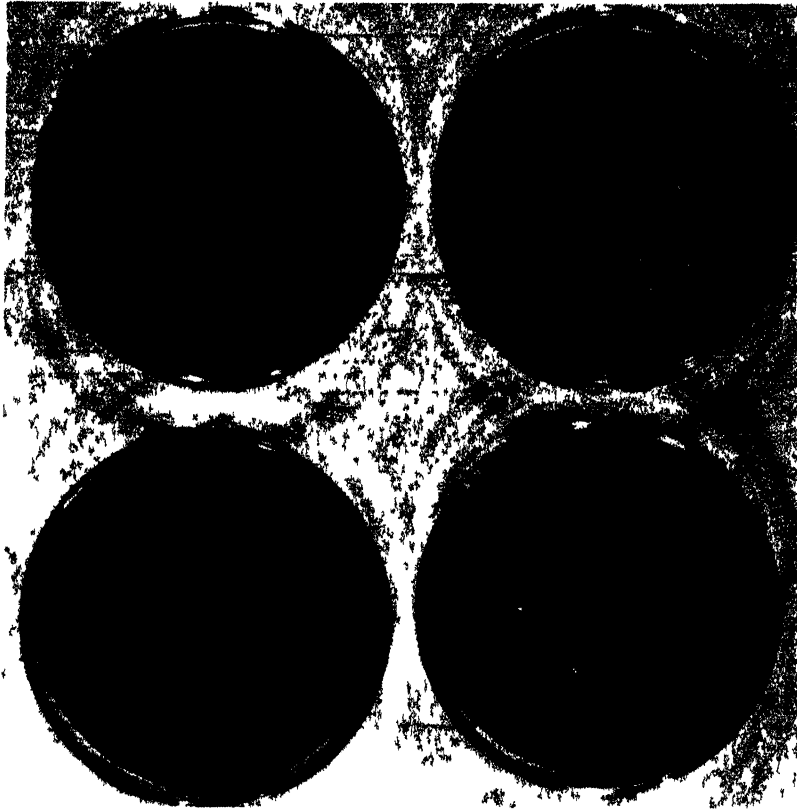
FIG 2   Germination of sclerotia of *Sclerotium rolfsii*   Left, after 4 days' exposure to Larvacide in soil at 62° F , right, untreated. Upper plates, wet soil, lower, dry soil.

range of chloropicrin has not been determined for *Heterodera marioni*. Newton, Bosher, and Hastings (8) concluded that 1 ml of chloropicrin would kill *Ditylenchus dipsaci* at 6 inches while 2, 4, and 6 ml. did not kill all the nematodes at 8 inches   In our tests the bags of inoculum were 7½ inches from the point of injection, a distance that may have been too great for effective kill with chloropicrin.

Another reason for ineffectiveness of chloropicrin may have been the use of unrotted nematode galls   Rotting of galls appears to be necessary for chloropicrin to be effective (12).   On the other hand, rotting of galls

TABLE 1.—*Effect of temperature on the efficacy of Larvacide against Heterodera marioni, Fusarium oxysporum f. callistephi, and Sclerotium rolfsii*[a]

Soil treatment	Exposure (in days)	Temperature °F.						
		52	53	57–59	72	82	98	Totals
Relative root-knot index[b]								
Larvacide	1	3.0	2.8	2.5	3.0	2.8	0.0	14.1
	3	3.3	3.3	3.0	2.8	2.5	0.5	15.4
	6	3.0	2.8	2.3	1.5	2.0	0.5	12.1
	9	3.5	3.5	3.0	3.0	3.0	0.0	16.0
	14	2.8	3.3	2.3	2.0	3.0	0.0	13.4
	Totals	15.6	15.7	13.1	12.3	13.3	1.0	71.0
None	1	3.7	4.0	3.7	4.0	4.0	4.0	23.4
	3	4.0	4.0	4.0	4.0	3.5	4.0	23.5
	6	4.0	4.0	4.0	4.0	4.0	3.0	23.0
	9	4.0	4.0	4.0	4.0	4.0	4.0	24.0
	14	4.0	4.0	4.0	4.0	4.0	4.0	24.0
	Totals	19.7	20.0	19.7	20.0	19.5	19.0	117.9
Numbers of dead aster plants[c]								
Larvacide	1	3	4	3	3	4	0	17
	3	4	2	4	3	0	0	13
	6	1	1	2	2	1	0	7
	9	0	1	1	1	0	0	3
	14	1	3	2	2	0	0	8
	Totals	9	11	12	11	5	0	48
None	1	4	4	4	4	4	3	23
	3	4	3	4	4	4	3	22
	6	4	4	4	4	4	0	20
	9 ·	4	3	3	4	4	0	18
	14	3	4	2	4	4	0	17
	Totals	19	18	17	20	20	6	100
Germination of sclerotia[d]								
Larvacide	1	60	100	86	21	39	20	326
	3	22	14	8	16	15	27	102
	6	15	12	4	2	6	5	44
	9	2	5	6	0	0	54	67
	14	4	0	2	0	2	10	18
	Totals	103	131	106	39	62	116	557
None	1		100	93	97	98	100	488
	3	100	100	100	95	95	99	589
	6	100	97	100	96	99	87	579
	9	97	100	100	100	87	80	564
	14	90	72	64	63	52	52	393
	Totals	387	469	457	451	431	418	2613

[a] Efficacy is based on the viability of these organisms and is tested as follows: relative root-knot index on squash plants, survival of aster plants,. and germination of sclerotia, respectively.

[b] 0.0 represents complete kill of the nematodes and no galling; 4.0 represents little or no kill and maximum galling.

[c] 4 dead plants represents no reduction in viability, and 0 dead plants represents complete kill, of the Fusarium.

[d] Each figure represents the number of sclerotia germinating out of 100 tested.

does not appear to be necessary when methyl bromide is used, for Stark and Lear (12) reported that methyl bromide diffused through the soil and killed nematodes in non-rotted galls in 2 hours.

There was a uniform reduction in nematode population at all the lower temperatures in Larvacide-treated soil as compared with the nematode population in the untreated soil at the same temperatures. Apparently the full effect of the Larvacide occurred the first day; no further kill of the nematode was apparent after 3 days or more. Complete kill of the Fusarium occurred at 98° F. after 1 day's exposure and nearly complete kill at 82° after 3 days' exposure. However, a temperature of 98° had a detrimental effect on the Fusarium in the untreated soil, and the combined effect of high temperature and Larvacide may have been responsible for the complete kill at this temperature after 1 day. The reason for the detrimental effect of the high temperature is not clear since this temperature is much below the

TABLE 2.—*Effect of soil moisture on the efficacy of Larvacide against Fusarium oxysporum f. callistephi as indicated by the numbers of dead aster plants*[a]

Exposure (in days)	Soil treated with Larvacide		Soil not treated	
	Wet soil[b]	Dry soil[c]	Wet soil[b]	Dry soil[c]
1	7	10	11	12
3	7	6	11	11
6	3	4	10	10
9	0	3	10	8
14	1	7	8	9
Totals	18	30	50	50

[a] Twelve dead plants represents no reduction in viability, and 0 dead plants represents complete kill, of the Fusarium.
[b] The moisture level of the wet soil was maintained at 17 to 20 per cent.
[c] The moisture level of the dry soil was maintained at 7 to 10 per cent.

thermal death point of most fungi, including Fusarium (6). The effect was less at 72° or lower. At these lower temperatures a longer time was required for effective fungicidal action. Germination of the sclerotia of *Sclerotium rolfsii* was nearly 100 per cent in each of the untreated lots with the exception of those remaining in the soil for 14 days. The majority of these latter sclerotia were in poor condition and had been attacked by other fungi and bacteria which were not identified. Larvacide reduced the viability of these sclerotia at temperatures of 72°, 82°, and 98° after 1 day and at all temperatures after 3 days. Nearly complete kill occurred at all temperatures after 6 days. The discrepancy (Table 1) at 98° after 9 and 14 days could not be accounted for. In another test the number of germinating sclerotia was greater after 14 days' exposure to chloropicrin than similar sclerotia exposed for 6 or 10 days. Godfrey and Young (3) killed sclerotia of *Sclerotium rolfsii* with chloropicrin by using 3.17 ml. per cubic foot of soil in the sealed containers. The temperature was not given. This is approximately the same concentration we used but our treatments were in unsealed containers.

As shown in table 2, Larvacide was more effective against the Fusarium in wet soil than in dry soil, but it was slightly better against *Sclerotium rolfsii* in dry than in wet soil. In two experiments, of 4,500 sclerotia treated in dry soil, 1,138 germinated, and of 4,500 sclerotia treated in wet soil, 1,380 germinated. Davey and Leach (2), however, reported that wet sclerotia of *S. rolfsii* were killed by a 24-hour exposure to chloropicrin, but dry sclerotia were not. Because the root-knot galls apparently were beyond the effective killing range of the Larvacide for this organism in unrotted galls, no information was obtained on the relation between soil moisture and nematode kill.

D-D. Nematode kill was complete after 1 day's exposure to D-D in the soil at 82° to 98° F. and after 3 days' exposure at 62° and 72° (Table 3). D-D was relatively ineffective against *Fusarium oxysporum* f. *callistephi*, although the results were obscured by the fact that the oat medium on which

TABLE 3.—*Relative root-knot index[a] on squash plants following exposure of Heterodera marioni to D-D at different soil temperature and moisture levels*

Exposure (in days)	Soil Temperature (° F.)						Wet soil[b]	Dry soil[c]
	46–54	55	62	72	82	98		
1	3.3	3.5	3.5	3.5	0.0	0.0	2.1	2.5
3	4.0	4.0	0.0	0.0	0.0	0.0	0.8	1.3
6	2.8	2.5	0.0	0.0	0.0	0.0	0.5	1.0
9	2.3	2.1	0.0	0.4	0.0	0.0	0.4	1.2
13	2.9	2.3	0.0	0.8	0.0	0.0	0.4	1.5
Totals	15.3	14.4	3.5	4.7	0.0	0 0	4.2	7.5

[a] 0.0 represents complete kill of the nematodes and no galling; 4.0 represents no kill and maximum galling.
[b] The moisture level of the wet soil was maintained at 17 to 20 per cent.
[c] The moisture level of the dry soil was maintained at 7 to 10 per cent.

the fungus was growing fermented badly in the D-D treatments, particularly at the highest temperature (98°). This fermentation did not occur in the other treatments. Because of the fermentation it is questionable to what extent the D-D was responsible for the reduction in viability of the Fusarium; 164 out of a total of 240 aster plants from all D-D treatments were killed, which indicates that the majority of the fungus samples were still viable. In any event, the fungicidal effect was low.

Dowfume G. As in the case of Larvacide, the nematode galls apparently were beyond the effective range of Dowfume G and, although a slight reduction in nematode viability did occur, little effect of temperature or moisture was evident (Table 4). This material was least effective against the nematodes at the highest temperature (90° to 101° F.), contrary to the results with the other fumigants; but there were no differences in effectiveness between the other temperatures. It may be that at temperatures of 90° to 101° the Dowfume G was dissipated from the soil so rapidly that it had no chance to affect the root-knot galls at such a distance.

There was a reduction in germinability of the sclerotia after 6 days' exposure in the Dowfume G-treated soil (Table 4). This reduction was most rapid at the higher temperatures but was not affected by soil moisture. Apparently this reduction was due to the action of antagonistic organisms on the sclerotia, since neither methyl bromide nor ethylene dichloride, constituents of Dowfume G, has been considered to be noticeably fungicidal

TABLE 4.—*Effect of soil temperature and moisture on the efficacy[a] of Dowfume G and of Dowfume D-15 against Heterodera marioni and Sclerotium rolfsii*

Soil treatment	Exposure (in days)	Soil temperature °F.						Totals	Wet soil[d]	Dry soil[e]
		61–70	61–71	64–72	72–80	90–86	90–101			
		Relative root-knot index[b]								
	1	3.0	3.0	3.0	3.5	3.3	4.0	19.8	3.6	3.0
	3	2.5	3.0	2.5	2.8	2.8	4.0	17.6	3.3	2.6
Dowfume G	6	3.5	2.3	2.3	2.8	2.3	4.0	17.2	2.7	3.0
	9	2.5	2.3	2.5	2.5	2.0	3.0	14.8	2.1	2.9
	14	2.5	2.5	2.3	2.0	2.0	3.3	14.6	2.1	2.8
	Totals	14.0	13.1	12.6	13.6	12.4	18.3	84.0	13.8	14.3
	1	3.0	3.0	2.3	2.5	2.0	1.8	14.6	2.8	2.1
	3	0.0	0.5	0.5	0.5	0.8	0.0	2.3	0.0	0.8
Dowfume W-15	6	0.5	0.5	0.5	0.5	1.5	0.8	4.3	0.0	1.4
	9	1.3	1.0	1.0	1.0	1.5	0.5	6.3	0.2	1.9
	14	0.8	1.0	1.3	1.0	1.0	0.0	5.1	0.0	1.7
	Totals	5.6	6.0	5.6	5.5	6.8	3.1	32.6	3.0	7.9
		Germination of sclerotia[c]								
	1	100	100	100	100	100	100	600	300	300
Dowfume G	6	100	95	94	20	35	6	350	151	199
	13	75	67	10	0	21	2	175	95	80
	Totals	275	262	204	120	156	108	1125	546	579
	1	100	100	100	89	99	99	587	289	298
Dowfume W-15	6	94	100	47	59	46	2	348	138	210
	13	51	31	32	0	7	0	121	16	105
	Totals	245	231	179	148	152	101	1056	443	613

[a] Efficacy is based on the viability of these organisms and is tested as follows: relative root-knot index on squash plants and germination of sclerotia, respectively.
[b] 0.0 represents complete kill of the nematodes and no galling; 4.0 represents no kill and maximum galling.
[c] Each figure represents the number of sclerotia germinating out of 100 tested.
[d] The moisture level of the wet soil was maintained at 17 to 20 per cent.
[e] The moisture level of the dry soil was maintained at 7 to 10 per cent.

(3), except at high concentrations (7), and most of the sclerotia that failed to germinate were badly disintegrated. Viability of *Fusarium oxysporum* f. *callistephi* was not affected by this material.

Dowfume W-15. This material was effective against nematodes at all temperatures (Table 4). Some reduction in viability was evident after

24 hours' exposure particularly at the highest temperature. No further reduction in viability occurred after 3 days, however. This material was more effective in wet than in dry soil.

Dowfume W–15, like Dowfume G, was not effective against *Fusarium oxysporum* f. *callistephi* but did reduce the viability of sclerotia of *Sclerotium rolfsii*. The greatest reduction occurred at 90° to 101° F., and there was considerable reduction at 64° to 86° after 6 days. Further reduction in viability occurred after 13 days at all temperatures, but how much of this effect was due to the fumigant and how much due to antagonistic organisms is not known. The greatest reduction of viability occurred in moist soil.

Retention of the fumigants in the soil. As mentioned previously, test bags of the organisms were buried in the center of the soil in the cans 1, 3, and 6 days after the introduction of the fumigant, and were removed 14

TABLE 5.—*Effect of moisture on the retention of Larvacide, Dowfume G, and Dowfume W-15 in the soil as indicated by the survival of Fusarium oxysporum f. callistephi and Heterodera marioni introduced into the soil 1, 3, and 6 days after the introduction of the fumigant*[a]

Test organism added after:	No. of dead aster plants[b]				Relative root-knot index[c]			
	Soil treated with Larvacide		Soil not treated		Soil treated with Dowfume G		Soil treated with Dowfume W-15	
	Wet[d]	Dry[e]	Wet[d]	Dry[e]	Wet[d]	Dry[e]	Wet[d]	Dry[e]
1 day	0	4	10	11	2.2	2.9	0.0	2.2
3 days	5	11	10	11	2.3	2.3	0.0	3.2
6 days	11	11	10	11	2.6	2.8	0.8	2.9
Totals	16	26	30	33	7.1	8.0	0.8	8.3

[a] All test organisms were removed 14 days after the introduction of the fumigant.

[b] 12 dead plants represents no reduction in viability, and 0 dead plants represents complete kill, of the organism.

[c] 0.0 represents complete kill of the nematodes and no galling; 4.0 represents no kill and maximum galling.

[d] The moisture level of the wet soil was maintained at 17 to 20 per cent.

[e] The moisture level of the dry soil was maintained at 7 to 10 per cent.

days after the introduction of the fumigant and tested for viability. This was to provide information on the retention of the fumigant in the soil in sufficient quantity to reduce viability of the test organisms. Data are given in tables 5 (effect of moisture) and 6 and 7 (effect of temperature) for Larvacide, Dowfume G, and Dowfume W-15. Data were not obtained for D–D, and *Sclerotium rolfsii* was not used in these tests.

Larvacide was effective in wet soil for a longer time than in the dry soil (Table 5). There was a sufficient amount remaining in the wet soil after 1 day to kill the Fusarium introduced at that time. It was slightly effective after 3 days in the wet soil but not in the dry soil, and was not effective after 6 days in either the wet or dry soil. Dowfume G was relatively ineffective after 1 day in either the wet or dry soil, although the root-knot

TABLE 6.—*Effect of temperature on the retention of Larvacide in the soil as indicated by the survival of Fusarium oxysporum f. callistephi introduced into the soil 1, 3, and 6 days after the introduction of the fumigant*

Fungus added after:	Number of dead aster plants[b] at						Totals
	52°F.	53°F.	58°F.	72°F.	82°F.	98°F.	
1 day	0	1	2	1	0	0	4
3 days	2	4	2	2	2	4	16
6 days	4	3	4	3	4	4	22
Totals	6	8	8	6	6	8	42

[a] All test organisms were removed 14 days after the introduction of the fumigant.

[b] Four dead plants represents no reduction in viability; 0 dead plants represents complete kill of the Fusarium.

index was slightly less in wet than in dry soil. Dowfume W-15 was effective in the wet soil for a much longer time than in the dry soil. A sufficient amount of the fumigant was present after 3 days to give complete kill of *Heterodera marioni*, and almost complete kill occurred when the bags of nematode galls were buried in the soil 6 days after the introduction of the fumigant.

The effects of temperature on the retention of Larvacide are given in table 6, and for Dowfume G and Dowfume W-15 in table 7. Larvacide was still effective after 1 day regardless of temperature and was slightly effective after 3 days at temperatures of 52°, 58°, 72°, and 82° F., but not at 53° and 98°. Its ineffectiveness after 3 days at 98° was expected, but no satisfactory explanation for its ineffectiveness at 53° after 3 days can be given.

Dowfume G (Table 7) was not effective at 98° F. after 1 day but there was a slight effect at all the other temperatures even when the root-knot

TABLE 7.—*Effect of temperature on the retention of Dowfume G and Dowfume W 15 in the soil as indicated by the survival of Heterodera marioni introduced into the soil 1, 3, and 6 days after the introduction of the fumigant*

Fungus added after:	Relative root knot index[b] at						Totals
	61–70°F.	61–71°F.	64–72°F.	72–80°F.	80–86°F.	90–101°F.	
	Dowfume G						
1 day	2.3	2.5	2.8	1.8	2.0	4.0	15.4
3 days	2.0	2.3	2.3	2.0	2.0	4.0	14.6
6 days	2.8	2.3	2.5	2.3	2.5	4.0	16.4
Totals	7.1	7.1	7.6	6.1	6.5	12.0	46.4
	Dowfume W-15						
1 day	0.8	1.0	1.0	1.0	1.0	1.8	6.6
3 days	1.8	1.8	1.8	1.3	1.0	2.0	9.7
6 days	1.3	1.8	1.3	1.8	2 0	3.0	11.2
Totals	3.9	4.6	4.1	4.1	4.0	6.8	27.5

[a] All test organisms were removed 14 days after the introduction of the fumigant.

[b] 0.0 represents complete kill of the nematodes and no galling; 4.0 represents no kill and maximum galling.

galls were buried in the soil 6 days after the introduction of the fumigant. The ethylene dichloride constituent of the Dowfume G may be responsible for the reduction in viability of *Heterodera marioni* after this long period. It has nematocidal properties as well as a much higher boiling point than methyl bromide (3) and would be expected to remain in the soil longer and be less affected by temperature than methyl bromide. Dowfume W-15 was most effective against the nematodes after 1 day at 52° F. and least effective at 98°. Fairly effective concentrations remained in the soil at temperatures of 52° to 58° for 6 days, but not at 98°.

<center>DISCUSSION</center>

Stark and Lear (12) have pointed out that there are limitations to greenhouse tests with soil fumigants. Such limitations are recognized in the tests reported herein. Nevertheless, it is believed that such tests are necessary for the proper evaluation of the effects of temperature and moisture on the efficacy of soil fumigants. Because of conflicting reports on the efficiency of soil fumigants over a wide range of temperatures, it is necessary that tests be conducted for determining the effects of temperature and moisture. This knowledge is particularly essential now that fall application of soil fumigants in northern areas is being advocated (1). If it can be shown that soil fumigants can be used effectively in the fall after crops have been harvested and when soil temperatures are low, many farmers would be willing to reclaim their soil by such treatment. When soil is treated in the fall the fumigant should be dissipated by spring so that crops could be sown early in the season.

The tests reported herein bear on this question, and, although far from adequate, they do indicate that fumigants may be effective at lower temperatures, although the action is slow. Larvacide without a water seal was surprisingly effective in these tests. Furthermore, it was retained for 1 to 3 days in wet, low-temperature soils in sufficient concentration to be toxic to *Fusarium oxysporum* f. *callistephi*. Many more tests of this type are needed, particularly for the low temperature ranges and for different soil types, before fumigation of low-temperature soils can be recommended.

<center>SUMMARY</center>

The efficacy of Larvacide, D-D, Dowfume G, and Dowfume W-15 against *Heterodera marioni* (Cornu) Goodey, *Fusarium oxysporum* f. *callistephi* (Beach) Snyder and Hansen, and *Sclerotium rolfsii* Sacc. at two soil moisture levels and six soil temperatures varying from 45° to 101° F., was investigated.

Larvacide was most rapidly effective against all three organisms at 98° F. It was only slightly effective against *Heterodera marioni* at the lower temperatures.

D-D was not effective against *Fusarium oxysporum* f. *callistephi* or *Sclerotium rolfsii*, but was effective against *Heterodera marioni*, causing complete kill at 82° or 98° F. after 1 day and at 62° or 72° after 3 days. *Heterodera marioni* was affected only slightly by D-D even after 3 days or more at 45° or 55° F.

Dowfume G showed no effect against *Fusarium oxysporum* f. *callistephi* and only slight effect against *Heterodera marioni*. There was a considerable reduction in viability of the sclerotia of *Sclerotium rolfsii* after 3 days at 72°, 82°, or 98° F. This may have been caused by the action of antagonistic organisms.

Dowfume W-15 was not effective against *Fusarium oxysporum* f. *callistephi* but was effective against *Sclerotium rolfsii* at 72°, 82°, or 98° F. after 6 days. It was most effective against *Heterodera marioni* at 98° after 1 day and after 3 days was effective at all temperatures.

In general, the fumigants were most effective in wet soils, and they were retained longest in the wet, low-temperature soils.

PLANT INDUSTRY STATION

BELTSVILLE, MARYLAND

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THREE INTERSPECIFIC HYBRIDS OF TOBACCO

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Interspecific hybrids of *Nicotiana tabacum* L. with three other species of *Nicotiana* are recorded in this paper. They were attempted primarily for their possible resistance to downy mildew or blue mold (*Peronospora tabacina* Adam), blackshank (*Phytophthora parasitica* Dastur var. *nicotianae* Tucker), and root knot (*Heterodera marioni* Goodey). It is thought that they may be of interest (a) for the methods used and the types of hybrids produced, (b) for possible genetic, cytological, and chemical studies, and (c) for further breeding to introduce disease resistance and perhaps other desirable characteristics into various commercial types of tobacco.

The pistillate parent of each hybrid was of the commercial cigar-wrapper (shade) tobacco variety Rg, originated at this Station (8), and highly resistant to blackshank in the field in Florida. Without artificial shade, it commonly grows to a height of 8 feet, and produces about 26 leaves.

The staminate parents were of the species *Nicotiana debneyi* Domin., *N. repanda* Willd. *ex* Lehm., and *N. plumbaginifolia* Viv. The seeds were originally obtained from the Division of Tobacco, Medicinal, and Special Crops, United States Department of Agriculture. Resistance of these species to several diseases is reported in the literature available. *N. debneyi* is highly resistant to downy mildew (4), and also resistant to black root rot and wildfire (5). *N. repanda* is highly resistant to root knot (3, 5), and to black root rot and wildfire (5), and also resistant to blackshank (6). *N. plumbaginifolia* is highly resistant to downy mildew (4); it is also resistant to root knot, the nematodes from tomato entering the roots but developing only slightly and producing no eggs (2). In field tests at Quincy, Florida, *N. repanda* and *N. plumbaginifolia* were moderately resistant to blackshank.[1]

Several attempts to produce the hybrids described in this paper are reported in the literature. Kostoff, in a critical review published in 1941, described his sterile hybrids of *Nicotiana tabacum* and *N. debneyi* (9, p. 698), and reviewed a report by Pal and Nath on their sterile *N. tabacum* × *N. plumbaginifolia* hybrids (9, p. 656). No report of a fertile cross between *N. tabacum* and *N. repanda* has been found in the literature, but Foster (6) obtained seedlings of *N. repanda* (4-N) × *N. tabacum* (2-N) which failed to recover after transplanting. Clayton (4) reported successful crosses of *N. tabacum* with *N. debneyi* and *N. plumbaginifolia*.

The methods used in this work were conventional, except that the method of mixed pollination (1) was used in some attempts. A small amount of

[1] *N. megalosiphon* Huerk and Muell. is reported resistant to downy mildew (4) and to root knot (2, 3), and at Quincy, Florida, was moderately resistant to blackshank. Two unsuccessful attempts have been made at this Station to produce the hybrid, *N. tabacum* × *N. megalosiphon*, but Clayton (4) reported having made this cross.

self pollen was applied to the stigma along with a large amount of foreign pollen to insure the setting of the capsule, although no great difficulty was experienced without this precaution. All F_1 plants were examined for the purpose of detecting hybrids.

Each F_1 hybrid was transplanted into a 4-gallon jar and grown to maturity either in the greenhouse or outdoors. The identifying number was assigned when the first hybrid seeds were produced.

Nicotiana tabacum × N. debneyi

This cross was attempted once in 1940 without self pollen, with no success. It was attempted again in 1941 and 1942, using mixed pollination. The 1942 attempt (No. 798) yielded three seedlings which appeared to be hybrids. After transplanting, they grew slowly to heights of from $7\frac{1}{2}$ to $9\frac{1}{2}$ feet, produced from 41 to 54 small leaves, and bloomed from 2 to 4 months later than normal for the *Nicotiana tabacum* parent. One of them is shown in figure 1. All were nearly self-sterile, but set seed readily with pollen of *N. tabacum*. All produced fertile F_2 progeny, and were resistant to blackshank. No. 798–3 was of fairly good cigar-wrapper type.

The progenies of 798–1 and 798–3 had no appreciable resistance to downy mildew in the plant bed, but possessed some resistance to downy mildew leaf spotting in the field. This was apparently associated with a characteristic which they have in common with *Nicotiana debneyi*; that is, moisture (such as rainwater) spreads in a thin film over the surface of the leaves and hence evaporates quickly. Leaves of the Rg variety of tobacco collect large drops of water and remain wet for a considerably longer time. The quick-drying characteristic may be of value in connection with resistance to other leaf diseases, and possibly of agronomic value. No. 798–2, which did not have the quick-drying characteristic, was not tested for downy mildew resistance in the plant bed; it had no resistance in the field.

It is an interesting coincidence that Chileno Correntino, a tobacco variety introduced from Argentina (T. I. 57), moderately resistant to downy mildew (7), also has the quick-drying characteristic. This suggests the possibility that Chileno Correntino may have originated as a cross between *Nicotiana tabacum* and *N. debneyi*.

In crosses between tobacco varieties Rg and Chileno Correntino, the quick-drying characteristic appeared to be due to a single dominant factor.

Nicotiana tabacum × N. repanda

This cross was attempted three times without self pollen, with no success. The 1941 attempt (No. 784), using mixed pollination, yielded one seedling which appeared to be a hybrid. It grew rather slowly to a height of 6 feet, produced 48 small leaves, and bloomed 2 months later than normal for the *Nicotiana tabacum* parent. It produced a large crop of viable seed. The F_2 (Fig. 2) and succeeding generations were highly resistant to blackshank; some lines were moderately resistant to root knot. The leaves were thick and of no value as cigar wrappers.

Nicotiana tabacum × *N plumbaginifolia*

Of four attempts made to accomplish this cross without self pollen, three yielded one hybrid plant each, all self fertile.

No 853 (1945) grew normally in the greenhouse during the winter, and resembled the *Nicotiana tabacum* parent rather closely.   The F_2 and later

Fig 1 (*Left*).   *N. tabacum* × *N debneyi* hybrid, F_1 (No 798).
Fig. 2 (*Right*).   *N tabacum* × *N repanda* hybrid, F_2 (No 784–1).

progeny were resistant to blackshank, and some F_4 lines appeared resistant to root knot in 1948.

No 871 (1946) grew slowly, produced a moderate number of leaves, and bloomed 2 months later than normal for the *Nicotiana tabacum* parent. Plants of the F_2 generation in 1948 were large and coarse, and rather susceptible to blackshank, but were apparently resistant to root knot.

No 870 (1946) grew very slowly, produced a large number of leaves, and bloomed 4 months later than normal for the *Nicotiana tabacum* parent.

Plants of the F_2 generation in 1948 had a large number of medium-size, thick leaves, and short internodes. All of the 1948 crop died of blackshank.

No. 870 in the F_2 generation closely resembled the White Honduras variety of tobacco in the small size of seeds and slow growth of seedlings, the appearance of the plants, and the extreme susceptibility to blackshank. Considered together with the root-knot resistance of both White Honduras (7) and *Nicotiana plumbaginifolia* (2), these similarities suggest the possibility that White Honduras originated as a cross between *N. tabacum* and *N. plumbaginifolia.*

In summary, this paper records the production of fertile hybrids between *Nicotiana tabacum* L., cigar-wrapper variety Rg, as the pistillate parent, and *N. debneyi, N. repanda,* and *N. plumbaginifolia.* All of the hybrids resembled the *N. tabacum* parent much more closely than they did the other parent. Their resistance to certain tobacco diseases and some other characteristics are recorded.

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DIFFERENTIATION OF TOBACCO AND TOMATO RING-SPOT VIRUSES BY CROSS IMMUNIZATION AND COMPLEMENT FIXATION[1]

M. G. TALL, W. C. PRICE, AND KENNETH WERTMAN

INTRODUCTION

Serological reactions and cross immunity tests have proved extremely useful for differentiating plant viruses. When used for this purpose, the results of the two methods have generally been in strict accord (1, 2, 3, 4, 5). However, in a few cases (5, 11, 12, 13) the relationships among plant viruses indicated by serological studies have not agreed with those indicated from cross immunity tests. Price (10) showed by cross immunity tests that tomato ringspot virus was distinct from tobacco ringspot virus. Chester (5), on the other hand, found by precipitin tests that these two viruses had similar antigenic structures.

In view of the seemingly contradictory evidence reported by Price and Chester, experiments were made to re-examine the serological and cross immunological relationships of the two viruses. As a further control on the validity of the methods, a new undescribed strain, here designated as the Pennsylvania strain of tobacco ringspot virus or more simply as Pennsylvania ringspot virus, was included in the experiments.

The methods chosen were the plant cross immunity and complement fixation tests. Although Chester (4) did not obtain a successful complement fixation test with tobacco ringspot or tomato ringspot viruses, nor has one been reported in the literature so far as can be determined by the authors, the complement fixation technique used here proved both sensitive and specific.

MATERIALS AND METHODS

The plant viruses used were as follows:

(a) Tobacco ringspot virus, *Annulus tabaci* H. var. *virginiensis* H., has been described in detail by Fromme, Wingard, and Priode (7), and by Wingard (17). It was obtained originally from Dr. Wingard.

(b) Tomato ringspot virus, *Annulus zonatus* H., was originally isolated and described by Price (10), who designated it tobacco ringspot virus No. 2. The name was later changed and reported in Holmes' (9) handbook.

(c) The Pennsylvania strain of tobacco ringspot virus, *Annulus tabaci* H. var. *Pennsylvanicum* n.v., was first seen on flowering tobacco plants in the horticultural gardens at Pennsylvania State College. The symptoms and behavior of this virus on tobacco plants are similar to those of the type strain. However, in this host it differs from the type in being somewhat

more infectious, in showing somewhat more rapid movement, and in usually producing slightly larger zonate necrotic lesions. Evidence for close relationship between the Pennsylvania and type strains will be discussed under the experimental section.

Stock cultures of the viruses were maintained in Turkish tobacco plants in a greenhouse that was fumigated at frequent intervals to control insects.

Methods of Inoculation

Except where otherwise noted, the rubbing method of inoculation was used. Cheesecloth pads were moistened with a virus preparation and gently rubbed over the surface of test leaves. For mass transfers, an infected leaf was wrapped in a square of cheesecloth and macerated by pounding with a pot label, and the resulting substance was rubbed over the leaves. Immediately after inoculation the leaves were sprinkled with water. In special cases inoculations were carried out by cleft grafting.

Host Plants

Turkish type tobacco (*Nicotiana tabacum* L.), Holmes' (8) necrotic-type hybrid (*N. digluta* Clausen and Goodspeed × *N. tabacum* L.), and Bountiful bean (*Phaseolus vulgaris* L.) were used as host plants. All plants were grown in composted soil in 4-inch pots in the greenhouse. Symptoms produced in Turkish type and necrotic-type tobacco plants were indistinguishable. Bountiful bean plants infected with either the type or Pennsylvania strain of tobacco ringspot virus developed necrotic, ringlike, local lesions and systemic necrosis within five days. The viruses soon thereafter involved the terminal bud and resulted in death of the plant. Bountiful bean plants infected with tomato ringspot virus showed necrotic, ringlike, local lesions similar to those produced by tobacco ringspot virus. They either failed to show evidence of systemic infection or developed zonate systemic lesions in from seven to ten days after inoculation. Terminal buds were killed in only a few instances. The variability in movement of tomato ringspot virus within bean plants is comparable to that observed in Turkish tobacco plants (10).

Cross Immunity Tests

Turkish tobacco and necrotic-type tobacco plants were inoculated when they had reached a height of about six inches. Such plants were held on a greenhouse bench until they had recovered and were then used as a source for tip cuttings. Cuttings were rooted in a mixture of moist peat moss and sand and then transferred to composted soil in 4-inch pots. When they had reached a height of about six to eight inches, they were used as test plants in all cross protection experiments.

Complement Fixation Tests

Since viruses used in complement fixation tests were not completely purified, antigens used to immunize rabbits were prepared from Turkish

tobacco plants while those used to react with antisera were prepared from Bountiful bean plants. This was done on the assumption that the antigenic constituents of normal tobacco plants were serologically distinct from those of normal bean plants, an assumption which later was shown to be correct.

Except where otherwise mentioned, the procedure for preparing antigens was as follows. Leaves inoculated ten days earlier and showing numerous necrotic spots were surface-sterilized for ten minutes in a 1 : 10,000 $HgCl_2$ solution. The leaves were then passed through four sterile saline washes and crushed aseptically under a hood in a mortar containing sterile alundum. The crushed tissue was spun in an angle centrifuge for thirty minutes at approximately 2500 rpm. A turbid green supernatant extract was obtained, poured off, and frozen at $-20°$ C. for 24 to 36 hr. The fluid extract was thawed and spun again for 30 min. at 2500 rpm. Following this centrifugation, a clear, straw-colored supernatant fluid was obtained. The antigen in this form was pooled, thoroughly agitated, and dispensed in sterile serum bottles in 5-ml. lots. The antigens were then labeled and stored at $-20°$ C. until needed.

Preparation of Antisera. Approximately 25 ml. of blood was taken aseptically from each of four rabbits by means of cardiac punctures. Each of the four rabbits was then injected with antigen preparations containing normal tobacco sap, tobacco ringspot virus, Pennsylvania ringspot virus, and tomato ringspot virus, respectively. A total of seven intraperitoneal injections in about 4-ml. amounts were administered to each rabbit. One week after the last injection, approximately 25 ml. of blood was taken aseptically from each rabbit by cardiac puncture.

Normal sera and immune sera were separated aseptically from blood samples in the following manner: Each sample of blood was allowed to clot for one hour at room temperature, then rimmed and stored overnight at 10° C. The blood was then spun in an angle centrifuge, and the supernatant serum removed. Each serum was dispensed in 1-ml. lots, labeled, and stored at $-20°$ C. until needed. All sera used in antigen and antiserum titrations were inactivated at 56° C. for 30 min.

Preliminary antigen titrations indicated that the tomato ringspot antiserum contained no antibodies demonstrable by the complement fixation tests used. In view of this finding, two additional 7-ml. injections were administered over a two-week period. The rabbit was bled seven days after the final injection, and the antiserum was separated as previously described. As will be seen later, not even the two additional injections of tomato ringspot antigen was sufficient to raise the demonstrable antibody titer to a significant level. Consequently the virus was concentrated about twenty-five times by high speed centrifugation and the concentrate injected into the same rabbit. These injections proved effective in inducing specific complement-fixing antibodies.

The complement fixation technique used in these experiments has been

described in detail by Westman (16).   Commercial Antisheep Hemolysin Glycerinized obtained from Sharp and Dohme and sheep erythrocytes obtained from Brown Laboratory, Topeka, Kansas, were used in preparing the hemolytic system.   Complement was obtained from Sharp and Dohme under the trade name Lyovac Complement.

RESULTS OF CROSS IMMUNITY TESTS

Cross immunity tests were performed to determine whether or not tobacco plants that had recovered from any one of the three ringspot virus diseases would be immune from either of the other two ringspot diseases. For this purpose, one set of cross protection tests was carried out on Turkish tobacco plants and another set was carried out on necrotic-type hybrid plants.

Sets of cuttings each containing three or more plants grown from healthy Turkish and necrotic-type tobacco plants and from plants that had recovered from tobacco ringspot, Pennsylvania ringspot, and tomato

TABLE 1.—*Summary of cross protection tests with recovered plants of Turkish and necrotic-type tobacco*

Virus used for inoculation	Plant recovered from			
	Tobacco ringspot	Pennsylvania ringspot	Tomato ringspot	Controls
Tobacco ringspot	0	0	+	+
Pennsylvania ringspot	0	0	+	+
Tomato ringspot	+	+	0	+

0 indicates complete protection.
+ indicates no protection.

ringspot viruses, respectively, were challenged in all possible combinations with the three viruses.   The challenge inoculation was made by rubbing in one set of experiments and by cleft grafting in another.

Results of these experiments were identical and are summarized in table 1.   Plants that had recovered from tobacco ringspot virus developed no lesions when reinoculated with the same virus or with Pennsylvania ringspot virus.   Similarly, plants that had recovered from Pennsylvania ringspot virus showed no lesions when reinoculated with the same virus or with tobacco ringspot virus.   On the other hand, plants that had recovered from either Pennsylvania or tobacco ringspot virus developed lesions when reinoculated with tomato ringspot virus.   Plants that had recovered from tomato ringspot virus showed no lesions when reinoculated with the same virus, but developed lesions when inoculated with either the Pennsylvania or tobacco ringspot viruses.

Because of the complete protection between tobacco ringspot and Pennsylvania ringspot virus, it can be concluded that these viruses are closely related strains belonging to the same virus group.   The failure of these

two virus strains to protect against tomato ringspot virus, together with the failure of tomato ringspot virus to protect against the two strains of tobacco ringspot virus, indicates that these viruses are not closely related strains and belong in separate virus groups.

RESULTS OF SEROLOGICAL STUDIES
Activity and Sterility Tests

Activity tests were conducted with each virus antigen to determine the extent of active virus present in the preparation. Dilution end points were determined by rubbing dilutions of $10^{0.5}$, $10^{-1.0}$, $10^{-1.5}$, $10^{-2.0}$, and $10^{-2.5}$ of the antigen preparation over test leaves of Turkish tobacco plants. Each dilution was tested on five leaves of each of four plants and the infections were recorded 15 days later. The dilution end point of antigens prepared from both Turkish tobacco and Bountiful bean plants was $10^{-2.0}$ for both tobacco and Pennsylvania ringspot virus, and $10^{-1.0}$ or less for tomato ringspot virus.

Bacterial plate counts on nutrient agar were made with each antigen to determine whether or not bacterial contamination would be sufficient to interfere with serological tests. Three ml. of a representative sample were plated in 10 ml. of nutrient agar and incubated at 37° C. Counts observed after 48 hr. incubation varied from none to five organisms per ml The data indicate that the number of bacteria present in the prepared antigens was too small to cause interference with the specific complement fixation tests.

Complement Fixation Tests

Experiments were carried out to determine whether the serological relationships between the three viruses would agree with the immunological relationships previously found. The plan of the experiments was to compare the three viruses on the basis of their antiserum dilution end-point for complete fixation of complement in the presence of homologous and heterologous antigens. Before this could be done it was necessary to standardize the amount of hemolysin to be used in all subsequent tests, to standardize the amount of complement to be used in antigen titrations, and to standardize the amounts of both complement and antigen to be used in serum titrations.

Hemolysin titration. Titration of hemolysin was performed by adding 0 25 ml. of 3 per cent suspension of washed sheep erythrocytes to 0.25 ml. of varying dilutions of hemolysin. The tubes were allowed to stand for 10 min. at room temperature and then 0.50 ml. of a 1 : 30 dilution of complement and 0.50 ml. of fresh saline (0.85 per cent) were added. The tubes were incubated at 37° C. for 30 min. in a water bath, and read. The tube with the highest dilution of hemolysin showing complete hemolysis of sheep red blood cells was taken as one MHD (minimal hemolytic dose) of hemolysin. Three MHD of hemolysin were used in complement, antigen, and

serum titrations. This system is referred to here and in later sections as sensitized red blood cells.

Complement titrations. Complement titrations were conducted before each antigen titration and before serum titrations. Titrations were carried out with varying quantities, *i.e.* 0.08, 0.10, 0.12 . . . 0.26 ml. of a 1:30 dilution of complement. The titration was made in the presence of 0.25 ml. of diluted antigen. The dilution of antigen used in complement titration prior to antigen titrations was 1:10. Thereafter the dilution of antigen used in complement titrations was comparable to the amount used in subsequent serum tests. Table 2 shows the amount of materials used in complement titration. Final readings were taken after incubation at 37° C. for 30 min. The first tube showing complete hemolysis of the sensitized red blood cells was taken as the exact unit and the tube with the next highest amount of complement as a full unit. Two full units contained in 0.50 ml. of fresh saline (0.85 per cent) were used in the antigen titrations

TABLE 2.—*Milliliters of various materials used in titration of complement*

Material	Tube number									
	1	2	3	4	5	6	7	8	9	10
Incubated at 37° C. for 30 min.										
Complement (1:30)	0.08	0.10	0.12	0 14	0.16	0.18	0.20	0.22	0.24	0.26
Saline (0.85 per cent)	0.67	0.65	0.63	0.61	0 59	0.57	0.55	0.53	0.51	0.49
Antigen[a]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Sensitized R.B.C.	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

[a] Dilution of 1:10 was used in titration prior to antigen titration. Dilution containing two antigenic units was used in titration prior to serum titration.

and in the final titration of sera. All dilutions in these and subsequent tests were made in fresh saline.

Antigen titration. Each antigen was titrated to find the highest dilution of antigen which, when mixed with homologous antiserum, would fix complement over a range of antiserum dilutions. This range should be wide enough to permit comparisons between the reactions of antigen with homologous and heterologous antisera in serum titrations. This dilution of antigen was designated as one antigenic unit. Two antigenic units were used in subsequent serum titrations.

The antigen titration was carried out by mixing 0.25 ml. of twofold serial dilutions of antigen with equal volumes of twofold serial dilutions of homologous antiserum in the presence of two units (0.50 ml.) of previously titrated complement. Normal serum controls were included as well as the usual antigen and antiserum controls. Twofold serial dilutions of normal bean antigen were mixed with the antiserum and normal serum and served as additional controls. The purpose of this was to determine whether or not the serum of rabbits immunized with virus extracts of tobacco plants would fix complement in the presence of extracts of normal bean plants. As will be seen later, no significant cross reaction occurred.

At the same time a second complement titration, in the absence of anti-serum and in the presence of each antigen, was carried out as a control. Varying amounts of diluted complement, *i.e.*, 0.10, 0.20, 0.25 (one full unit), 0.30, 0.35, 0.40, and 0.50 (two full units) ml. were mixed with 0.25 ml. of a 1:10 dilution of antigen. The dilution of complement used was equal to that found in the preliminary titrations. Fresh saline was added to all tubes in amounts necessary to bring the final volume to 1.50 ml. After a primary incubation for 18 hr. at 4°–8° C., 0.5 cc. of sensitized red blood cells was added to each tube which was then incubated for 30 min. at 37° C. and read. The purpose of this second complement titration was twofold: (1) to determine whether or not there had been any in-activation of complement during the primary incubation period, and (2) to check on the validity of the preliminary complement titration. Thus it served to demonstrate that all fixation of complement was due to anti-gen-antibody reaction.

The mixtures were incubated at 4°–8° C. for 15 to 18 hr. Following this, 0.50 ml. of sensitized red blood cells was added to each tube. The tubes were shaken, incubated in a water bath at 37° C. for 30 min., and read. The antigenic unit was recorded in terms of the dilution origi-nally added to the tubes. All antigen titrations were repeated several times, each repetition confirming the original findings.

Results obtained from titrations showed that a 1:15 dilution of both tobacco and Pennsylvania ringspot viruses contained two units of antigen. However, the titration of tomato ringspot virus indicated that complement-fixing antibodies were either absent from the antiserum or present in such low concentrations as to make subsequent serum titrations insignificant. For this reason, it was necessary to concentrate the virus by high speed centrifugation and to make additional injections with the concentrated material.

Serum titration. Tests were conducted to determine the dilution end-point for complete complement fixation of antiserum for each of the three viruses in the presence of two units of homologous and heterologous virus antigens. In these tests, 0.25 ml. of solution containing two units of antigen was mixed with 0.25 ml. of twofold serial dilutions of antiserum. All possible combinations of antigens and antisera were made. The di-lution of antiserum ranged from 1:12 to 1:768.

Three normal rabbit serum controls were included, as well as the usual antigen and antiserum controls. Dilutions of normal tobacco antiserum were mixed with the three antigens and served as additional controls. The purpose was to determine whether or not the serum of a rabbit immu-nized with normal tobacco sap would fix complement in the presence of virus antigens prepared from bean plants. Two full units of complement in 0.50 ml. were added to all mixtures of antigen and serum.

The mixtures of serum, antigen, and complement were incubated at 8° C. for 16 hr. Following this, 0.50 ml. of sensitized red blood cells was

added. The mixtures were then incubated in a water bath at 37° C. for 30 min. and final readings were made. End points were estimated on the basis of the last tube showing complete fixation of complement and the titer recorded as the dilution of serum originally added to that tube. As in the case of the antigen titrations, a second complement titration was made but in this case two units of each antigen were used. Again this served as a check on the preliminary complement titration and demonstrated that all fixation of complement was due to antigen-antibody reaction.

The data are summarized in table 3. The conclusions to be derived from these results are as follows:

(1) Normal antigenic constituents of Turkish tobacco plants differ from those of Bountiful bean plants since antibodies formed against nor-

TABLE 3.—*Summary of complement fixation tests*

Sera	Antigens			
	Normal bean sap 1 : 6	Tobacco ringspot virus[a] 1 : 15	Pennsylvania ringspot virus[a] 1 : 15	Tomato ringspot virus[a] 1 : 12
Normal rabbit No. 1	0[b]	0	0	0
do No. 2	0	0	0	0
do No. 3	0	0	0	0
Tobacco sap	0	0	0	0
Tobacco ringspot virus	0	1 : 384[c]	1 : 384	0
Pennsylvania ringspot virus	0	1 : 384	1 : 384	0
Tomato ringspot virus[d]	0	0	0	1 : 48

a Two antigenic units, in all cases prepared from infected bean plants.
b No fixation of complement
c Dilution of antiserum showing complete complement fixation.
d Serum prepared by injection of virus concentrated 25 times by high-speed centrifugation of infected tobacco plants.

mal tobacco sap did not react with either normal bean sap or with virus antigens prepared from bean plants.

(2) Antigen preparations of tobacco and Pennsylvania ringspot viruses induce the formation of demonstrable complement-fixing antibodies in rabbits.

(3) Because antisera prepared against tobacco and Pennsylvania ringspot viruses completely fixed complement to almost equal dilution endpoints with either virus antigen, it can be concluded that these viruses have similar antigenic structures. This would indicate that the two viruses are closely related strains and belong in the same virus group. The serological relationship is in complete accord with that indicated by cross protection tests.

Tests with Centrifuged Tomato Ringspot Virus

Preliminary results obtained with tomato ringspot virus suggested that the preparations used contained insufficient virus to induce antibody formation. Tests were therefore carried out with a tomato ringspot virus prepara-

tion that had been concentrated about 25 times by high-speed centrifugation.[2] Leaves from 69 infected tobacco plants were harvested and crushed with a mortar and pestle. The sap was extracted, centrifuged at 3,000 r.p.m. for 15 min., then frozen overnight at − 10° C., thawed and again centrifuged at low speed. Two hundred forty cc. of the clear green fluid supernatant was spun at 33,560 r.p.m. for 1½ hr. The supernatant from the centrifugation was discarded. The pellet was suspended in 10 cc. of sterile 0.85 per cent saline and centrifuged at 5,000 r.p.m. for 10 min. The clear, slightly greenish supernatant was used as an immunizing antigen. It produced numerous local lesions on tobacco plants at a dilution of $10^{-1.5}$ and a few lesions at $10^{-2.0}$.

The concentrated preparation of tomato ringspot virus was used to inject the same rabbit used previously for tomato ringspot virus immunization trials. The rabbit was injected intraperitoneally with 2 cc. of the concentrated antigen and 6 days later with 5 cc. of the same antigen. It was bled 8 days after the final injection. Complement fixation tests were carried out in the manner previously described, with the immune serum mixed with an antigen prepared from infected bean plants in the usual manner.

The data (Table 3) show that tomato ringspot virus, when sufficiently concentrated, is capable of inducing specific complement-fixing antibodies in rabbits. The failure of tomato ringspot virus antiserum to react with either the type or Pennsylvania strains of tobacco ringspot virus, together with the failure of tomato ringspot virus to react with antiserum for either strain of tobacco ringspot virus, strongly suggests that these two viruses are serologically unrelated.

DISCUSSION

The studies reported in this paper demonstrate that viruses of the tobacco and tomato ringspot groups induce the formation of demonstrable complement-fixing antibodies when injected into rabbits. The complement fixation technique used here proved sensitive and specific for both viruses. Chester (4) was unable to obtain a successful complement fixation test with tobacco ringspot virus, among others, finding that "In order for the reaction to be error-free, the immune serum and the virus extract must each be diluted to about 1 : 20. This double dilution lies so far beyond the point of precipitation with these viruses that even a complement fixation reaction is not obtained."

Data reported here show that a 1 : 15 dilution of tobacco ringspot virus completely fixed two units of complement in the presence of homologous antiserum diluted as high as 1 : 384. Likewise, a 1 : 12 dilution of tomato ringspot virus completely fixed two units of complement in the presence of homologous antiserum diluted 1 : 48. It is clear from this that the comple-

ment fixation technique used by Chester was not nearly so sensitive as the technique described in this paper. This may be due to the different primary incubation periods used in the two techniques. Chester's primary incubation period for serum, virus extract, and complement was 1 hr. at 37° C. The primary incubation period used in these experiments was 15 and 18 hr. at 4° C.

The greater sensitivity of the technique using cold primary incubation is due to a more complete antigen-antibody reaction, for it was shown by second complement titration that two full units of complement remained after the primary incubation. This eliminated the possibility of some complement being inactivated during the incubation period, leaving only minimal amounts to be fixed.

Wertman (15) reported the effect of primary incubation on normal egg antigens when titrated against syphilitic serum. Three different periods of incubation were employed, *i.e.*, 1 hr. at 37.5° C., 4 hr. at 4° to 8° C., and 18 hr. at 4° to 8° C. He found that the greater fixation occurred when the 18-hr. incubation in the cold was utilized. Damon and Johnson (6) also reported greater sensitivity with icebox primary incubation. They concluded that the greater sensitivity of the icebox technique was especially important in testing sera having minimal concentration of antibodies.

Chester's (4) complement fixation technique proved inadequate not only for tobacco ringspot virus, but also for the viruses of cucumber mosaic, potato veinbanding, and potato aucuba mosaic. This failure, together with the fact that twenty-one other viruses and virus strains have failed to respond to any sort of serological reaction (5), indicates that there is a decided need for sensitive serological techniques in plant virus research. Further studies may show that the sensitive complement fixation technique described in this paper will prove successful for determining the antigenic relationships of some of those other viruses, and thereby be of further use in plant virus classification.

The symptomatology and general characteristics of the Pennsylvania strain of tobacco ringspot virus suggest its close relationship to the type strain of tobacco ringspot virus. This relationship was definitely borne out by the serological and immunological data obtained. Therefore, it can be concluded that the Pennsylvania strain of tobacco ringspot virus belongs in the tobacco ringspot group of viruses.

Tomato ringspot virus differs from viruses of the tobacco ringspot group in host range and symptomatology (10). Immunological results reported here show that these two groups of viruses are not closely related. They confirm those of previous investigations (10, 14). Direct serological evidence concerning this relationship was obtained with antiserum produced by injection of a rabbit with a tomato ringspot virus preparation concentrated by ultracentrifugation. This evidence contradicts that of Chester (5) who obtained a cross-precipitin reaction between tobacco ringspot and tomato ringspot virus. It is generally agreed that the complement fixation

reaction is not only more sensitive, but more reliable than the precipitin reaction. We therefore conclude on the basis of our serological and immunological results and of previous immunological results (10) that tobacco ringspot and tomato ringspot viruses are not closely related.

SUMMARY

Tobacco ringspot virus and the heretofore undescribed Pennsylvania strain of tobacco ringspot virus elicit the formation of specific complement-fixing antibodies in the serum of rabbits. These antibodies can be demonstrated by the complement fixation technique described in this paper. Tomato ringspot virus likewise elicits the formation of specific complement-fixing antibodies when the virus in sap of diseased plants is concentrated at least twenty-five times by high speed centrifugation.

Dilutions of antiserum of tobacco and Pennsylvania ringspot viruses completely fix complement almost to the same dilution end-point with either the homologous or heterologous antigen. This indicates that the two viruses have similar antigenic structure and should be considered as strains belonging to the same virus group.

Tomato ringspot virus antiserum failed to react with either the type or Pennsylvania strain of tobacco ringspot virus. Conversely, antisera for neither the type nor Pennsylvania strain of tobacco ringspot virus reacted with tomato ringspot virus antigen. This result suggests that tobacco and tomato ringspot viruses are serologically unrelated.

Results of cross immunity tests with tobacco and Pennsylvania ringspot viruses were in accord with the serological results. Each virus protected tobacco plants completely against the other.

Results of cross immunity tests with tobacco and tomato ringspot viruses were likewise in accord with serological results. Neither virus protected tobacco plants against the other. It is concluded that the tomato and tobacco ringspot virus groups are immunologically and serologically distinct.

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NATURE OF SUSCEPTIBILITY TO HELMINTHOSPORIUM VICTORIAE AND RESISTANCE TO PUCCINIA CORONATA IN VICTORIA OATS[1]

S. C. LITZENBERGER[2]

INTRODUCTION

The oat variety Victoria (C.I. 2401) of *Avena byzantina* C. Koch., introduced from Uruguay by the United States Department of Agriculture in 1927, has been used extensively in breeding varieties resistant to crown rust, *Puccinia coronata* Corda. Selections from crosses with Victoria were found to be hypersensitive to the prevalent races of *P. coronata* so that cells immediately surrounding the infection loci collapse before the fungus can produce uredospores (Fig. 1). This type of resistance is so effective that Victoria has been used to develop the outstanding rust-resistant varieties Tama, Boone, Control, Vicland, Cedar, Vikota, Ventura, Osage, Neosho, Overland, Victorgrain, Lectoria and Lega. It has been estimated that 75 per cent of the oat acreage in 1945 in the major oat-growing sections of the United States was seeded to selections from crosses in which Victoria was one of the parents.

The popularity of these varieties is jeopardized by the widespread development on them of Helminthosporium blight caused by *Helminthosporium victoriae* Meehan and Murphy (2, 3), first described in 1946. Within the short span of two years it has been estimated that the acreage of the above varieties was reduced by about 10 per cent, and the reduction would have been much greater had seed of suitable substitute varieties been available.

Helminthosporium victoriae is regarded as highly specialized, since it has not caused injury to varieties other than those possessing the "Victoria type" of resistance to crown rust. Meehan and Murphy (2, 3) have reported that the secondary striping effect (Fig. 1) from *H. victoriae* is due to a toxin. The linkage between susceptibility to *H. victoriae* and resistance to *Puccinia coronata* is complete according to Murphy and

Meehan (4). The question thus arises as to whether these two reactions
are dependent upon the same mechanism.

It was considered desirable, therefore, to determine whether the two
fungi produced toxins which would induce similar injury to plants pos-
sessing hypersensitiveness to certain races of *Puccinia coronata* and com-
plete susceptibility to *Helminthosporium victoriae*. The results of several
experiments with these toxins are reported in this paper.
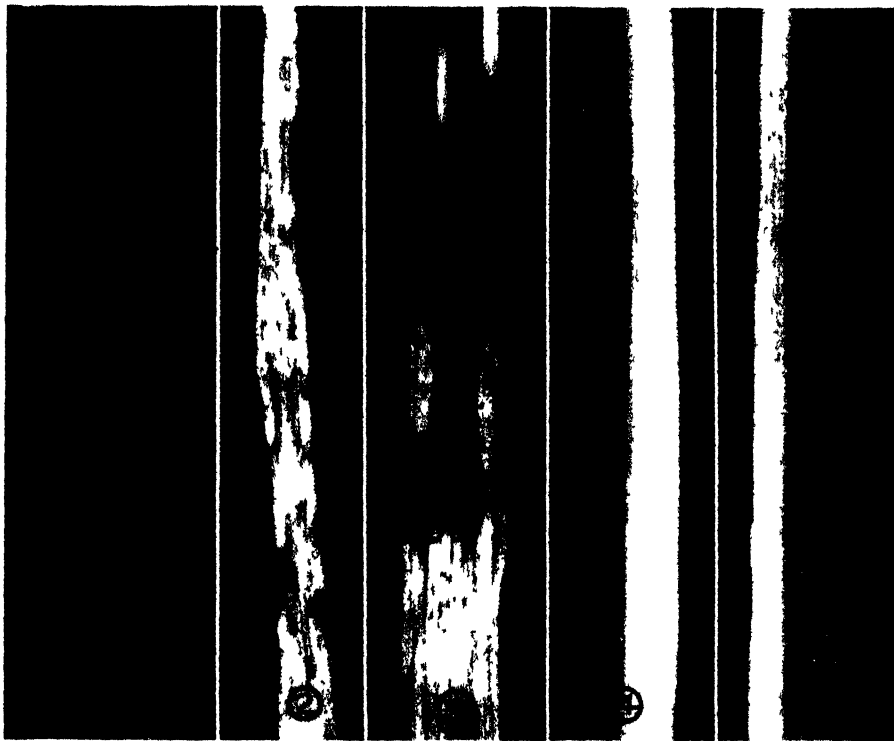
Fig. 1. Leaves of Victoria oats: 1 Normal. 2, 3. Necrosis resulting from infection
with *Puccinia coronata*. 4, 5. Striping resulting from *Helminthosporium victoriae*.

EXPERIMENTAL TECHNIQUES

At least two contrasting varieties of oats were used in each experiment.
In addition to a crown rust-hypersensitive and Helminthosporium blight-
susceptible variety (Victoria, Vicland, or Tama), a variety resistant to
Helminthosporium victoriae of Bond parentage (Bond or Clinton) was
used. In two tests a series of varieties was used for comparative pur-
poses.

The extracts containing the toxin of *Helminthosporium victoriae* were
obtained from pure culture of isolate II96 (1) and from greenhouse plants
inoculated with this culture. The cultures were grown on oatmeal agar in
Petri plates at room temperature (approx. 80° F.) for at least 10 days or
until the agar was completely covered with the developing fungus. The

mycelium from one Petri plate was scraped from the agar surface and macerated in 50 ml. distilled water in a Waring Blendor. For most experiments this mixture was steamed for 5 min. to kill the viable fungus and to facilitate release of the toxins. The concentrate was passed twice through Berkefeld filters to remove solid particles. The cleared extract, designated as the "basic concentrate," was then diluted with distilled water for testing.

In order to obtain comparable extracts, only seedlings having extensive basal infection were collected. The extracts were obtained either by subjecting the plants to 8,000 lb. pressure per sq. in. or by chopping them in a Waring Blendor with distilled water to liberate any toxins present. In either case the extract was steamed first and then passed through a Berkefeld filter. When difficulty was encountered in filtering, the solids were centrifuged out before filtering.

Toxins from crown rust were obtained by extracting plants infected with *Puccinia coronata* race 45. Infected leaves were harvested about 7 to 10 days after inoculation. At this time Victoria had not developed the pronounced necrotic flecks shown in figure 1, but Bond and other susceptible varieties had begun to produce sporulating pustules. Approximately 100 gm. plant material were diluted with 200 ml. distilled water in preparing the basic plant concentrate.

The effect on normal oat plants of the extracts from pure culture of *Helminthosporium victoriae* and also from infected plants was determined by several different methods. In preliminary tests the bloom was removed from the leaves of young oat plants, growing in pots, by passing them gently between moist fingers. Plants were sprayed with diluted extracts from various sources, placed in a moist chamber for about 8 hr. to retard drying and to permit maximum penetration, and then returned to the greenhouse. Observations on color, wilting, and necrosis were made several days later.

In other tests culms 4 to 18 in. long were cut at the ground line, re-cut under water, and inserted into the extracts. Usually the basic concentrate had been diluted with distilled water to several concentrations and about 20 ml. of each placed in a vial. A uniform number of cuttings ranging from 2 to 6 were placed in each vial in the different experiments. Final observations were made on wilting and necrosis within 4 days. In experiments where transpiration rates were measured, the vials were sealed with paraffin and weighed periodically.

The effect of the extracts on seedling development was determined by growing plants in soil extract agar and steamed or nonsteamed sand. The agar was prepared by a method employed by Dr. W. J. Hooker of the Iowa Agricultural Experiment Station, who has successfully used it in his studies with *Actinomyces scabies* (Thax.) Güssow. A 25-gm. sample of soil was added to a liter of tap water, allowed to stand for 24 hr., and decanted. The decanted liquid was made into 2 per cent agar medium,

sterilized and poured into 100-ml. beakers containing various concentrations of extracts. Seeds known to be free of disease organisms were dehulled, surface-sterilized in 10 per cent Clorox, and placed on the hardened agar. The beakers were covered with sterile Petri plates and kept at room temperature. The covers were removed when the growing seedlings began to be suppressed by them. Water was added as required after the seedlings began to develop. Although the method was not strictly aseptic, very few colonies of bacteria or fungi were observed. Similar methods were used with steamed and nonsteamed sand with due precautions against water-logging.

The treatments in the different experiments were arranged in randomized blocks whenever possible and replicated at least twice. The data for all major experiments were confirmed by repeated trials.

ABSORPTION OF EXTRACTS THROUGH LEAVES

Seedlings of Victoria and Bond that had attained a height of about 5 in. in 2-in. pots were de-waxed and sprayed with basic extracts from the culture of *Helminthosporium victoriae,* or from plants infected with either *H. victoriae* or *Puccinia coronata.* Suitable controls of each variety sprayed with water and healthy plant extract were included for comparison. The plants were placed in partial vacuum to encourage absorption of the spray deposit before they were placed in the humidity cabinet for slow drying.

All Bond plants remained normal during the 10-day period of observation irrespective of treatment. Severe injury was observed within 12 hr. on Victoria plants sprayed with extract from cultures of *Helminthosporium victoriae.* Necrotic lesions were numerous and during the first few days the leaves on severely affected plants dried. The final effect was very similar to the effect of a severe infection of crown rust on Victoria seedlings. Similar but less intense injury was observed on seedlings sprayed with extracts from plants infected with *H. victoriae.*

None of the injured plants died, as would have been expected if viable inoculum had been sprayed upon them. It was concluded, therefore, that the injury was due to a toxic substance or substances specific in effect on Victoria, as reported by Meehan and Murphy (3).

There was insufficient injury in this preliminary experiment to detect varietal difference in reaction to the extracts from plants infected with *Puccinia coronata.*

ABSORPTION OF EXTRACTS BY CUTTINGS

In order to obtain greater absorption of extract, a number of seedlings were cut and placed directly in portions of extracts. The plants were scored by visual observation into six classes, ranging from uninjured (Class 0) to complete yellowing, bronzing, and wilting (Class 5). Data on the effects of the various extracts are in table 1. They confirm obser-

vations in the preceding test except that the effects were more pronounced. Wilting of both Victoria and Bond cuttings was induced by the extracts from *Helminthosporium victoriae,* while characteristic streaking of leaves occurred only in Victoria cuttings. The presence of a toxic substance from *H. victoriae* in culture and from infected plants was fully confirmed. In addition, evidence was obtained of a mild toxic material from plants infected with *Puccinia coronata.* This material, as well as that obtained from plants infected with H. *victoriae,* also was specific to Victoria. No toxic effects on Bond were observed.

A second test was made with larger cuttings (about 18 in. tall) from Bond and Victoria. Duplicate plants of each variety were placed in 1 and 5 per cent concentrations of basic *Helminthosporium victoriae* extract, in extracts of *Puccinia coronata*-infected Bond and Victoria plants and of *H. victoriae*-infected Victoria plants. Results were approximately the same as those in the preceding experiment. The Bond variety was un-

TABLE 1.—*Effect of extracts from diseased and healthy oat plants and Helmintho-sporium victoriae on cuttings of Victoria and Bond oats*

Source of extract		Degree of damage[a] to cuttings	
Medium	Pathogen	Victoria	Bond
Victoria	None	1.3	1.3
Bond	None	2.0	1.5
Victoria	*P. coronata*	2.5	0.8
Bond	*P. coronata*	2.5	1.0
Victoria	*H. victoriae*	4.0	1.0
Culture	*H. victoriae*	5.0	2.0
Water	None	0.0	0.0

[a] Numerical rating: 0, uninjured; and 5, completely wilted and yellow.

affected except for slight wilting when placed in the more concentrated extracts (Fig. 2). The Victoria cuttings, on the other hand, were completely wilted and etiolated by *H. victoriae* extracts and were wilted appreciably by extract from *P. coronata*-infected plants. Victoria cuttings in water were unaffected, while cuttings in extract from healthy plants were only moderately affected.

In a third test, cuttings from 13 commercially important varieties possessing the hypersensitive type of resistance to *Puccinia coronata* and susceptibility to *Helminthosporium victoriae* of Victoria were compared with three *H. victoriae*-resistant varieties for reaction to extracts from diseased and healthy oat leaves. Cuttings from these varieties were also placed in water and a one per cent extract from *H. victoriae* for comparison. The cuttings were scored on a visual basis as described previously (Table 2).

Extracts from *Helminthosporium victoriae* and from leaves infected with this pathogen were specifically toxic to the cuttings of the 13 *H. victoriae*-susceptible varieties. They were scored 4.1 in *H. victoriae* extract and 4.8 in the extract from the diseased plants. The cuttings in water

were unaffected (Score 0) while those in the healthy plant extract aver-
aged 2.2.   The *H. victoriae*-resistant varieties had no symptoms of toxicity
beyond that obtained from the healthy plant extract.
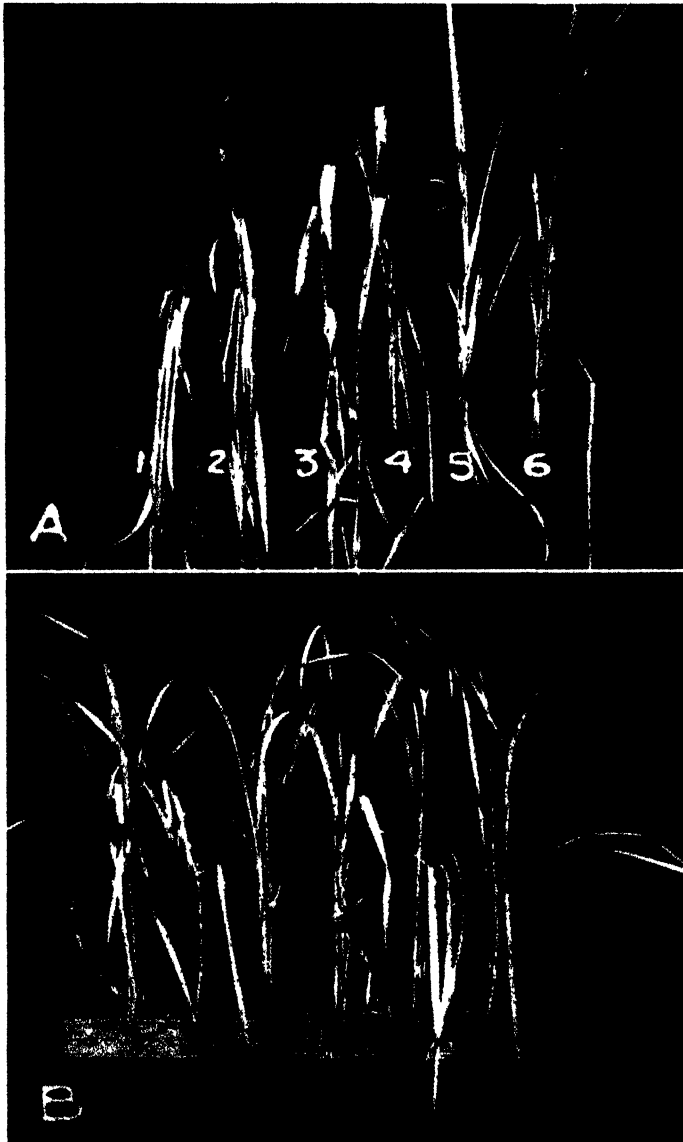
FIG. 2.   (A) Victoria and (B) Bond oat cuttings after 48 hours in extract solutions:
1. A 5 per cent dilution of *Helminthosporium victoriae* culture.   2. A 1 per cent dilution
of *H. victoriae* culture.   3. *H. victoriae*-infected plants.   4. *Puccinia coronata*-infected
plants.   5. Healthy plants.   6. Water.

The toxicity patterns with extracts from *Puccinia coronata*-infected
and healthy leaves were similar to those obtained with *Helminthosporium
victoriae* except that the differences were not so great nor so constant.

TABLE 2.—*Effect of extracts from Helminthosporium victoriae culture, Puccinia coronata- and H. victoriae-infected and healthy oat leaves on cuttings of 16 oat varieties*

Variety and reaction to H. victoriae	Degree of damage to cuttings when placed in extracts from									
	Water check	H. victoriae culture	Victoria leaves		Bond leaves		Gopher leaves		Marion leaves	
			Infected by H. victoriae	Healthy	Infected by P. coronata	Healthy	Infected by P. coronata	Healthy	Infected by P. coronata	Healthy
Susceptible										
Victoria	0	4	5	2	3	1	2	1	2	1
Vicland	0	4	5	3	3	2	2	2	3	2
Osage	0	5	5	2	5	1	2	2	3	2
Neosho	0	3	5	2	4	2	1	0	1	1
Overland	0	5	5	2	4	1	2	2	2	2
Stanton	0	3	5	2	5	2	3	1	3	2
Traveler	0	4	5	2	3	1	2	1	3	2
Fultex	0	5	5	3	3	2	2	2	3	2
Victorgrain	0	5	4	2	2	1	2	2	3	2
Beacon	0	3	4	2	3	2	2	2	3	2
Quincy Gray	0	4	5	2	4	3	2	1	2	1
Quincy Red	0	4	4	2	2	1	2	1	3	1
Garry	0	4	5	2	3	2	3	3	3	2
Av.	0	4.1	4.8	2.2	3.4	1.6	2.1	1.6	2.6	1.7
Resistant										
Marion	0	1	2	3	2	3	3	2	2	3
Bond	0	1	1	2	2	1	3	1	2	2
Gopher	0	2	2	3	4	3	3	3	4	3
Av.	0	1.3	1.7	2.7	2.7	2.3	3.0	2.0	2.7	2.7

ᵃ Numerical ratings: 0, uninjured; and 5, completely wilted and tissue collapse extensive.

Damage to cuttings from varieties possessing the hypersensitive resistance to crown rust of Victoria in extracts from healthy Bond leaves was consistently less or about one-half that in extracts from leaves infected with *P. coronata*. The extracts from leaves of Gopher and Marion infected with crown rust furnished less striking evidence of a toxic substance in the *P. coronata*-infected plants. Apparently any toxic substance which might have been present in these two varieties was in a lower concentration than in the Bond variety. Differential lodging may have been responsible for the different results obtained. Greater metabolic activity of the pathogen would be expected in the erect Bond plants than in the lodged Gopher and Marion plants.

TITER REQUIREMENTS FOR INJURY BY
HELMINTHOSPORIUM VICTORIAE EXTRACT

In order to determine the minimum active dosage of extracts from *Helminthosporium victoriae* with cuttings, a basic concentrate was prepared from pure culture and diluted with distilled water to 0, 5, 10, 25, 50, 75, and 100 per cent concentrations (Series I). In another series (Series II) 0, 0.5, 1, 2, . . . 14, and 15 per cent dilutions were prepared to determine the lower dosage range. Bond and Victoria cuttings were placed in each concentration. Leaves of Victoria plants in dilutions more concentrated than 25 per cent began to wilt in about 20 hours. Simultaneously, localized areas of leaf tissues collapsed. The rapidity of wilting was to a great extent correlated with concentration as was also the rapidity of streaking and yellowing or bronzing. The symptoms appeared much more rapidly at higher concentrations. Victoria cuttings succumbed in all dilutions including the 0.5 per cent concentration.

No important color change was observed in Bond cuttings. Wilting was observed in the cuttings, especially in the more concentrated solutions, but not to the extent observed in the Victoria cuttings. Wilting was correlated with amount of material absorbed, but no direct measurements were made to determine the exact amount. It also was noted that less solution was absorbed by both varieties as the concentration of the extract was increased.

The symptoms observed in the lowest dilutions were those generally observed under natural conditions, especially with older plants, indicating that the toxin concentration in cells of older plants affected would be at the dilution of about 0.5 per cent of the basic concentration of *Helminthosporium victoriae* culture. Concentrations above this point undoubtedly are responsible for the darkening of the tissue before bronzing or yellowing can become effective.

EFFECT OF HELMINTHOSPORIUM VICTORIAE
EXTRACT ON TRANSPIRATION

In previous experiments it had been observed that not all dilutions of extracts from *Helminthosporium victoriae* were absorbed equally by Vic-

toria and Bond cuttings. In order to determine how much effect the ex-
tract had on absorption or transpiration, uniform lots of culms (4 to 6
weighing 10 gm.) were inserted through holes in cork stoppers into flasks
containing 0, 5, 50, and 100 per cent dilutions of the basic concentration.
They were then sealed with cotton and paraffin to prevent loss of water
by evaporation and placed under three 200-watt Mazda bulbs for observa-
tion. At frequent intervals the flasks were weighed to determine the
amount transpired.

A summary of results under these favorable conditions for transpira-
tion are presented graphically for the 0, 5, and 100 per cent dilutions in
figure 3. The substance or substances contained in the filtrate produced
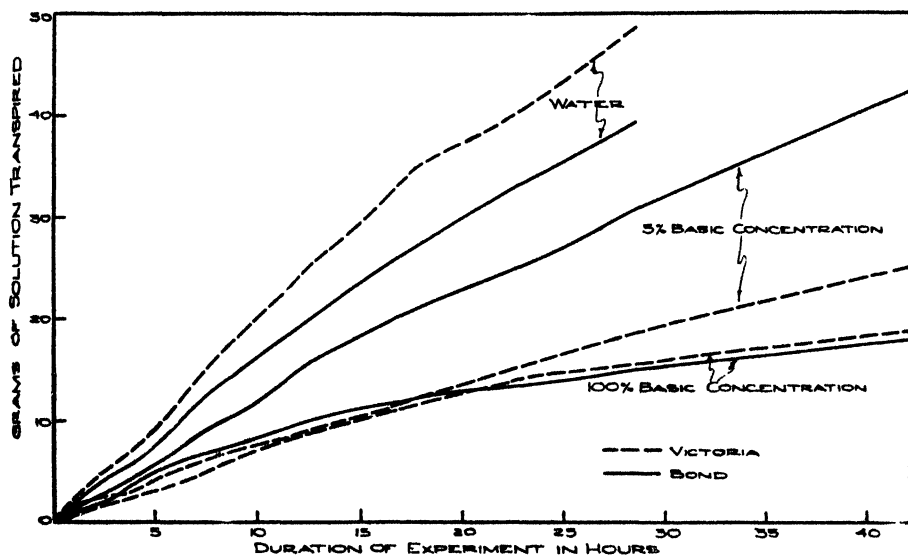
FIG. 3. Effect of extracts from *Helminthosporium victoriae* on transpiration of
Bond and Victoria oat cuttings.

two distinct effects upon the plant cuttings: (1) a reduction in transpira-
tion of both varieties and (2) rapid wilting with chlorosis and ultimate
death of the Victoria variety. Depression of transpiration rate in the oat
cuttings was generally in proportion to the amount of basic extract con-
tained in the solution. The reduced transpiration was also associated with
visual wilting. Wilting was more apparent with Victoria cuttings than
with Bond because of the extensive injury to the tissues.

The injurious effect was accompanied by loss of rigidity of all tissues
of the susceptible variety so that they appeared as though frozen. The
appearance of this symptom and rapidity of death were correlated with
the concentration of the basic extract. In the concentrated extract, wilt-
ing and chlorosis were observed in less than 5 hours, while in the 50 per
cent dilution similar symptoms were observed in 6 hours.

To study the effect of extract on transpiration at low and high humidi-

ties, an experiment similar to the previous one was set up. No special attempt was made to reduce the humidity below that prevailing in the laboratory (about 55 per cent), while a humidity of about 90 per cent was maintained in a large germination chamber.

Results are recorded in table 3, and representative flasks are in figure 4 of the experiment. At a humidity of 55, the results were essentially the same as those obtained in the previous experiment, transpiration in both varieties being depressed more or less in proportion to the concentration of the extract present in the absorbed solution. Cuttings kept in the high humidity chamber transpired only about one-seventh as much as those kept at low humidity. The transpiration trends, however, were similar.

TABLE 3.—*Effect of different concentrations of Helminthosporium victoriae culture extracts on transpiration of Victoria and Bond oat cuttings under favorable and unfavorable conditions for transpiration*

Time of exposure	Transpiration from plants in							
	Water		1 per cent basic conc.		5 per cent basic conc.		100 per cent basic conc.	
	Clinton	Tama	Clinton	Tama	Clinton	Tama	Clinton	Tama
(hr.)	*(gm.)*	*(gm.)*	*(gm.)*	*(gm.)*	*(gm.)*	*(gm.)*	*(gm.)*	*(gm.)*
	Low relative humidity with intense light (three 200-watt lamps)							
7	7.7	10 8	8.2	8.2	8 8	4.0	6.5	2.8
19	19.0	25.9	20.7	15.6	19.6	12.4	9.1	6.1
25	22.9	29.7	24.9	19.1	22.0	13.9	9.1	6.2
32	26.4	33.4	28.4	21.4	23.9	16.2	9.4	6.6
44	36.4	39.9	36.1	30.0	28.7	20.4	10.9	7.9
	High relative humidity with moderate light (one 200 watt lamp)							
3	0.90	0.90			0.95	0.50	0.80	0.65
11	2.70	2.25			2.23	1.43	1.87	1.57
18'	3.82	3.06			3.15	1.82	2.33	2.27
25	4.76	3.82			3.97	2.18	2.71	2.99
37	6.42	5.52			5.61	3.38	3.50	4.67
44	7.18	6.45			6.44	4.33	3.96	5.36

In either case treatments with higher concentrations of the extract reduced transpiration. Wilting, chlorosis, and death of cuttings of the susceptible Victoria variety developed more slowly at high humidity than under conditions less favorable for transpiration.

Because in the preceding experiments reduction in transpiration and subsequent wilting might have been the result of physical plugging of the vascular system at the base of the cuttings, seedlings of Clinton and Tama were cut and placed in extracts containing either 0, 5, or 100 per cent of the basic *Helminthosporium victoriae* concentration for either 5 or 10 hours. At the end of these periods, an additional one-fourth inch was cut off one-half of the culms in each group to remove any vascular obstructions that might have developed during the initial exposure. Then all plants were transferred to flasks of fresh tap water, sealed, and weighed. The
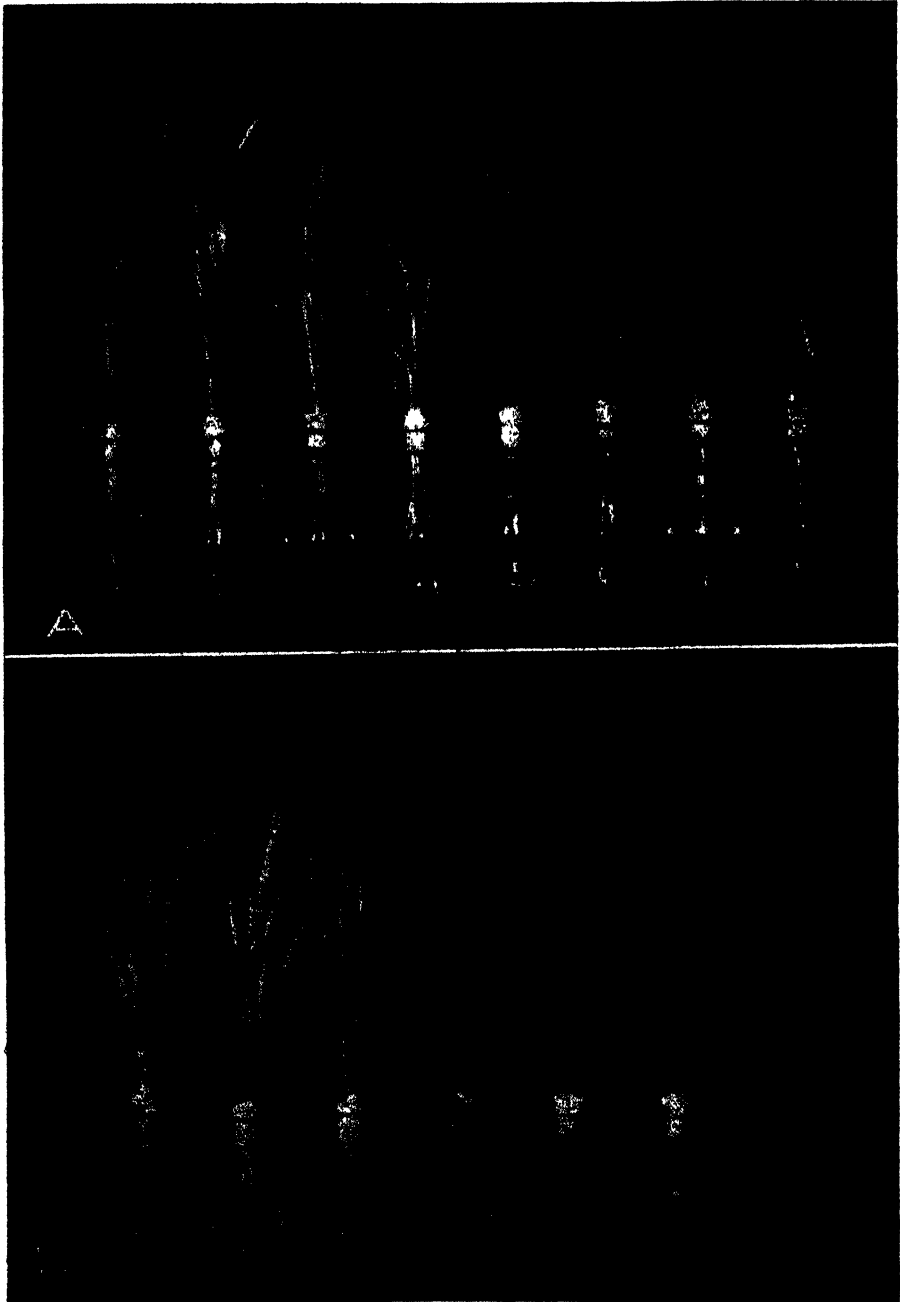
FIG. 4. Cuttings of Bond and Victoria in *Helminthosporium victoriae* extracts under low and high humidity conditions. A, low humidity: 1. Victoria in water. 2. Victoria in 1 per cent concentration. 3. Victoria in 5 per cent. 4. Victoria in 100 per cent. 5. Bond in water. 6. Bond in 1 per cent. 7. Bond in 5 per cent. 8. Bond in 100 per cent. B, high humidity: 1. Victoria in water. 2. Victoria in 5 per cent concentration. 3. Victoria in 100 per cent. 4. Bond in water. 5. Bond in 5 per cent. 6. Bond in 100 per cent.

losses in weight of the cuttings through transpiration were compared for recovery determinations.

Results from this study are in table 4. The *Helminthosporium victoriae*-susceptible Tama cuttings were damaged at both concentrations and they failed to recover when transferred to tap water. The leaves were killed with solutions containing the basic concentration and only the stems remained undesiccated. Injury to the cuttings resembled the immediate effects of frost injury. The 5 per cent dilution had less severe effects on the cuttings, primarily yellowing and bronzing and collapse of tissue in localized areas. Transferring cut and uncut culms to water did not affect

TABLE 4.—*Summary of transpiration data comparing cut and uncut stems of Clinton and Tama oats following initial treatment with extract from Helminthosporium victoriae*

Initial treatment		Basal treatment of stem after initial treatment	Transpiration from cuttings			
			Continuously in toxin extract		Removed to water	
Time of exposure	Material		Clinton	Tama	Clinton	Tama
(hr.)			(gm)	(gm)	(gm)	(gm.)
5	water	cut	10 38	12 83	11.76	14 71
		uncut	9.67	8 34	10.63	10.89
	5 per cent extract	cut	8 69	5 43	8 62	5.44
		uncut	9 06	4 88	7.38	4.00
	100 per cent extract	cut	4 74	4 84	2.41	4.41
		uncut	4 19	4.26	2.40	3.66
10	water	cut	18 73	24.21	16.46	26.02
		uncut	24 41	29.74	24 48	30.93
	5 per cent extract	cut	16 06	10 47	15 57	12.11
		uncut	18 01	8 93	13 31	8.43
	100 per cent extract	cut	7.13	7.58	2.84	5.43
		uncut	6.43	5 27	2.72	3.56

the transpiration rate, as initially established by the extract treatments.

The resistant Clinton cuttings, on the other hand, were injured much less severely by the extract. After treatment with the basic extract some localized areas of collapsed leaf tissue were observed. These plants did not recover from injury sustained in the initial extract treatment. The lower leaves remained wilted and the rate of transpiration remained at the reduced level even after transfer to water. This depression in Clinton cuttings indicated the injurious effects were from absorbed substances contained in *Helminthosporium victoriae* extract rather than from any possible basal plugging.

EFFECT OF EXTRACTS ON SEEDLINGS GROWING IN SOIL EXTRACT AGAR

Six seeds of Clinton and Vicland oats were transferred to hardened agar in each of several beakers. The soil extract agar contained dilutions

of 0, 5, 25, 50, and 75 per cent of the basic extract from *Helminthosporium victoriae* to study effects of the extract on germinating and developing seedlings. The stand was· later thinned to five plants per beaker.

Fresh root and top growth weights of the developing seedlings are reported in table 5. Representative plants from comparative treatments are shown in figure 5. Toxic material contained in the extract depresses root development as well as top growth, the depression being striking with the Vicland seedlings. The Vicland seedlings were practically destroyed in the 75 per cent dilution. The 5 per cent concentration was little better, where length of roots averaged 6 mm. and plant height 131 mm. as compared to the water check where root length was 165 mm. and top growth

TABLE 5.—*Effect of different concentrations of Helminthosporium victoriae culture extracts on developing seedlings of Clinton and Vicland in sterile soil extract agar*

Conc. of extract	Variety	Root length	Plant height	Fresh weights of plants		
				Roots	Tops	Total
(%)		*(mm.)*	*(mm.)*	*(gm.)*	*(gm.)*	*(gm.)*
None	Clinton	155	230	.18	.75	.93
(control)	Vieland	165	220	.20	.75	.93
5[a]	Clinton	85	275	.21	.88	1.09
	Vicland	7	132	.07	.36	.43
5[a]	Clinton	72	250	.25	.85	1.10
	Vicland	5	130	.07	.38	.45
25	Clinton	63	225	.33	.94	1.27
	Vicland	3	40	.03	.20	.23
50	Clinton	60	230	.30	.56	.86
	Vicland	2	30	.02	.14	.16
75	Clinton	45	210	.10	.71	.81
	Vicland	0	25	.01	.06	.07

[a] These two treatments differ in the preparation of the culture medium, the upper having been prepared with 50 per cent, while the lower was prepared with 95 per cent of soil extract agar.

was 220 mm. Fresh green weights were closely· correlated with physical measurements. The effect of the extract on Clinton was mild as compared to the effect on the *Helminthosporium victoriae*-susceptible Vicland. Only with the 50 and 75 per cent dilutions was there any appreciable reduction in growth of seedlings. The roots of Clinton, like those of Vicland in the water control, were normal for color, while Vicland roots in presence of the toxic extract were distinctly light brown.

In another experiment Clinton and Vicland were again grown in soil extract agar with extracts from *Puccinia coronata*-infected plants, *Helminthosporium victoriae*-infected plants, *H. victoriae* culture (5 per cent dilution), healthy plants, and water. Data on the various extracts (Table 6) confirm the observations in preceding trials with other methods. Tox-
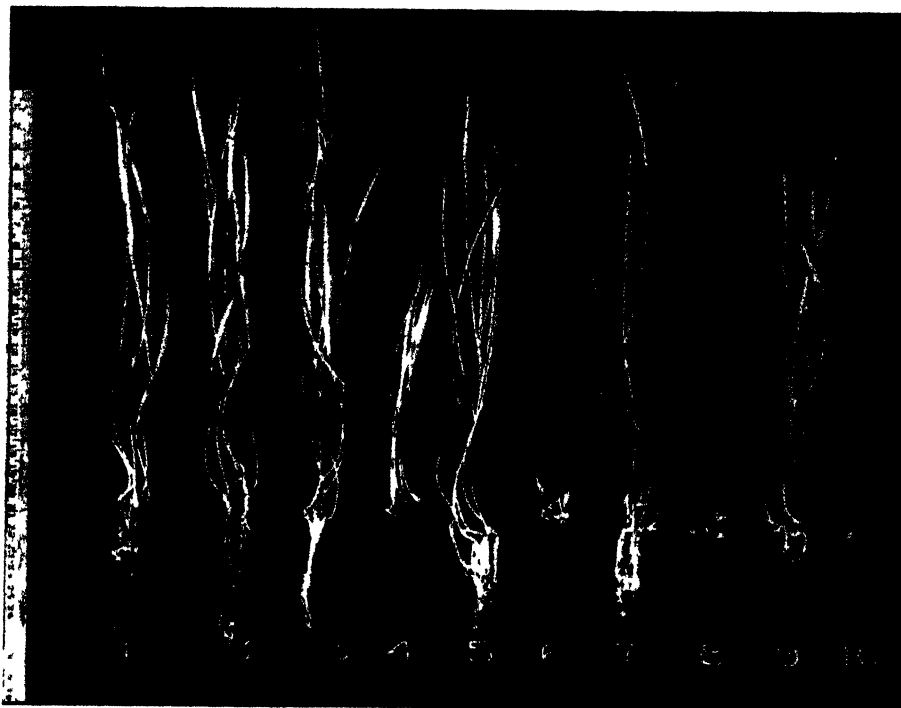
FIG. 5. Seedlings of Clinton and Vicland grown in sterile soil extract agar to which were added extracts from *Helminthosporium victoriae* culture. 1. Clinton in water. 2. Vicland in water. 3. Clinton in 5 per cent extract. 4. Vicland in 5 per cent. 5. Clinton in 25 per cent. 6. Vicland in 25 per cent. 7. Clinton in 50 per cent. 8. Vicland in 50 per cent. 9. Clinton in 75 per cent. 10. Vicland in 75 per cent.

icity or depression as evidenced by reduced root and top growth was obtained with Victoria seedlings from the *H. victoriae* culture extract as well as from extracts from plants infected with *H. victoriae* or *P. coronata*. The plant extracts appeared to depress growth in seedlings of both varie-

TABLE 6.—*Effect of extracts from Helminthosporium victoriae culture, H. victoriae-infected plants, Puccinia coronata-infected plants, and healthy plants on developing seedlings of Clinton and Vicland in sterile soil extract agar*

Source of extract	Variety	Root length	Plant height	Fresh weights of plants		
				Roots	Tops	Total
		(mm.)	(mm.)	(gm.)	(gm.)	(gm.)
None	Clinton	124	233	.35	.86	1.21
	Vicland	107	207	.30	.66	.95
Healthy plants	Clinton	23	167	.15	.51	.66
	Vicland	18	172	.14	.47	.61
P. coronata-infected plants	Clinton	20	167	.14	.47	.61
	Vicland	14	157	.10	.43	.53
H. victoriae culture	Clinton	93	250	.23	.85	1.08
	Vicland	28	172	.11	.38	.50
H. victoriae-infected plants	Clinton	26	182	.14	.61	.75
	Vicland	14	182	.10	.47	.57

ties, but with Vicland the healthy plant extract was consistently less damaging to developing seedlings than were extracts from diseased plants. It could, therefore, be concluded that in addition to the depressing effects of plant extracts a toxic principle operated in Victoria seedlings, as has been found by previous methods of comparison. Root development, probably the best index for measuring the prevalence of the toxin, was consistently less with the plant extract from diseased plants.

EFFECT OF EXTRACTS ON SEEDLINGS GROWING IN SAND

Cultures in sterile sand with extracts of *Helminthosporium victoriae* and *H. victoriae*-infected plants, *Puccinia coronata*-infected plants, healthy plants, and water were made with Clinton and Vicland. Care was necessary in applying water to prevent waterlogging. In table 7 results obtained with these different extracts are given.

TABLE 7.—*Effect of extracts from Helminthosporium victoriae culture, H. victoriae-infected plants, Puccinia coronata-infected plants and healthy plants on developing seedlings of Clinton and Vicland in unsteamed sand*

Source of extract	Variety	Root length	Plant height	Fresh weights of plants		
				Roots	Tops	Total
		(*mm.*)	(*mm.*)	(*gm.*)	(*gm.*)	(*gm.*)
None (control)	Clinton	100	225	.54	.72	1.26
	Vicland	110	210	.50	.75	1.25
Healthy plants	Clinton	75	210	.37	.86	1.23
	Vicland	70	220	.32	.84	1.16
P. coronata-infected plants	Clinton	75	205	.43	.82	1.25
	Vicland	75	175	.23	.58	0.81
H. victoriae culture (1 per cent conc.)	Clinton	85	230	.45	.69	1.14
	Vicland	70	220	.36	.74	1.10
H. victoriae culture (5 per cent conc.)	Clinton	100	240	.39	.80	1.19
	Vicland	75	200	.32	.68	1.00
H. victoriae-infected plants	Clinton	70	210	.34	.81	1.15
	Vicland	70	210	.32	.81	1.13

tained with these different extracts are given. Depression in developing Vicland seedlings as evidenced by reduced root and top growth was obtained with extracts from *P. coronata* and *H. victoriae.* The data confirm those obtained with the soil extract agar method except that differences were less striking. It is entirely possible that in the presence of soil organisms the toxicity of the extracts may be reduced.

In another experiment eight seeds each of Clinton and Vicland were grown in steamed and nonsteamed sand to which were added *Helminthosporium victoriae,* extracts from the mycelial growth, and water. Five grams of sterile Webster loam soil were mixed with the contents of each beaker to supply additional nutrient to the growing seedlings. The data in table 8 verify observations made previously.

When viable *Helminthosporium victoriae* was placed with seeds of Vicland and Clinton, no Vicland plants survived. They were killed either

before or after emergence. Some seedlings of Clinton failed to emerge. When examined they were alive but folded up without the protection of the coleoptile. This reaction had been observed in greenhouse tests with the viable organism.

EFFECT OF HYPODERMIC INJECTION OF EXTRACTS ON GROWING PLANTS

Portions of the extracts were injected by hypodermic needle, into numerous stems of growing plants of both Bond and Victoria. Subsequent readings on the Victoria plants gave positive readings with the basic concentration from *Helminthosporium victoriae*. The symptoms observed were characteristic streakings of the leaves above the point of injection as is normally observed under natural conditions. The extract from *Puccinia coronata*- and *H. victoriae*-infected plants gave only weak streaking

TABLE 8.—*Effect of Helminthosporium victoriae and 5 per cent extracts from it on developing seedlings of Clinton and Vieland oats in steamed and unsteamed sand*

Treatment	Variety	Root length	Plant height	Fresh weights		
				Roots	Tops	Total
		(*mm.*)	(*mm.*)	(*gm.*)	(*gm.*)	(*gm.*)
None (control)	Clinton	100	225	.54	.72	1.26
	Vieland	110	210	.50	.75	1.25
Extract in steamed sand	Clinton	57	190	.27	.74	1.01
	Vieland	30	148	.17	.56	.72
Extract in unsteamed sand	Clinton	85	228	.29	.79	1.08
	Vieland	110	210	.34	.75	1.09
Viable *H. victoriae* on seed at sowing	Clinton Vieland*	58	195	.41	.74	1.15

* No plants survived; death occurred before or after emergence. The root development varied in accordance with the development of the plumule.

symptoms on Victoria, while other treatments and other varieties resulted only in localized necrotic lesions where the needle penetrated.

HYPODERMIC INJECTION AND NEEDLE DEPOSITION OF
VIABLE HELMINTHOSPORIUM VICTORIAE MYCELIUM

Viable mycelial suspensions were injected into oat culms by hypodermic needle. When stems thus inoculated were placed in the moist chamber for incubation, infection occurred on both susceptible and resistant varieties. Very small quantities of inoculum were necessary; in fact, puncturing the stem with the needle of the filled syringe seemed to be sufficient. Although infection was obtained in both varieties, only Victoria stems were killed by the pathogen. Only the tissues in the immediate vicinity of the point of inoculation or those above the point of infection were killed. To kill the entire plant, infection must occur in a more vital part than above the soil line on one of the tillers.

Uniform amounts of mycelium were inserted into oat stems with an or-

dinary dissecting needle to study movement of the toxic substance produced by the fungus. Infection was obtained in both resistant and susceptible varieties, but only tissues of the susceptible variety were destroyed by the toxin produced by the pathogen. In no case was there any apparent downward movement of the toxic substance nor was there any apparent movement into other tillers of the same plant except where the point of inoculation was very close to the ground line so that the fungus could penetrate the crown region of the plant.

Resistant varieties (Bond, Clinton, Ukraine, Iogold, Hajira × Joanette, and Markton) tested by this extreme method were not affected by the toxic substance produced by *Helminthosporium victoriae* even though the pathogen could be observed growing at the point of injection. In several instances individual stems died, but this apparently resulted from mechanical injury caused by the inoculating needle.

Stems of Victoria and Bond were inoculated with mycelium of several other species of *Helminthosporium*, but the effects were similar to those obtained with *H. victoriae* on varieties resistant to it.

DISCUSSION

Data have been presented in this paper to show that *Helminthosporium victoriae* produces a toxic substance (or substances) which reduces transpiration in both resistant and susceptible varieties of oats, and, in susceptible varieties, strikingly reduces growth and also destroys cells. The exact nature of this active material or toxin is not known, but its effects are so distinctive that there can be little doubt that it contributes to chlorosis and striping symptoms observed in plants infected with *H. victoriae*. The material is so potent that it is effective at very low dosages.

The presence of a toxin was demonstrated in diseased plant tissues at concentrations sufficient to affect the oat plant. There were some conflicting data on the plant extracts, since extracts from normal plants also yield injurious materials. However, infected plants yielded extracts with toxic effects consistently greater than those of extracts from normal plants.

The present concept is that the active ingredient contributes materially to the parasitism of *Helminthosporium victoriae*. The fungus is a facultative saprophyte, so the presence of injured tissue undoubtedly stimulates its more extensive development.

Some evidence was obtained of the presence of a comparable toxic substance in plants infected with *Puccinia coronata*. It cannot be maintained that the two fungi produce identical toxins, since neither has been purified. The toxins behave in a comparable fashion, however, as shown by a number of different trials with extracts from these two organisms.

The evidence indicates that the toxic substance from *Puccinia coronata* is released at about the time the fungus begins to sporulate. This coincides with the period when the host undergoes a hypersensitive reaction and sufficient cells collapse to restrict further development of the rust

fungus.   The release of metabolic by-products at this time is in keeping with observations of Welsh (5) with *Actinomyces*, a fungus which releases antibiotic substances at the time of sporulation.

The evidence strongly suggests that *Helminthosporium victoriae* and *Puccinia coronata* produce comparable toxins and that further parasitic development depends upon differences in the innate abilities of the two fungi.   The dead and injured cells probably serve as a readily available source of food for the facultative saprophyte and as a physiological barrier to an obligate parasite such as *P. coronata*.   If this reasoning is correct then the hypersensitive reaction to *P. coronata* can be attributed to the toxins produced.

Very strong supporting genetic evidence for the presence of a gene with pleiotropic effects has been obtained by the author in inheritance studies of crosses involving resistance and susceptibility to these two pathogens.   No plants were observed in thousands tested where the hypersensitive type of reaction to *Puccinia coronata* was separated from susceptibility to *Helminthosporium victoriae*.   Resistance to crown rust of the hypersensitive type possessed by Victoria always was associated with susceptibility to *H. victoriae* and is apparently dependent upon the gene for susceptibility to this pathogen for its expression.   In *H. victoriae*-susceptible germ plasm, resistance to crown rust of this type can be expressed, while in its absence plants are susceptible unless there are other factors present for resistance which inhibit the normal development of the crown rust pathogen.

CONCLUSIONS

1. Evidence was obtained that *Helminthosporium victoriae* in culture produces a toxic substance that, at very low dilutions, affects oat plants.

2. In Victoria plants the toxic extract of *H. victoriae* produces rapid chlorosis, wilting, and necrosis, the chlorosis and necrosis being specific to the susceptible genotypes.

3. The toxic extract reduces transpiration in both resistant and susceptible varieties.   Injured plants do not recover from this traumatic effect.

4. Toxins were detected from *H. victoriae*-infected plants in concentration sufficient to bring about effects similar to those obtained with the pure culture.

5. Extracts from plants infected with *Puccinia coronata* produced a similar effect on genotypes with a hypersensitive reaction.   The extracts from healthy plants also were toxic to these plants but the injury was not so severe.   It is believed that *P. coronata* produces a toxin in sufficient concentration by the time of sporulation to induce the localized necrosis described as a hypersensitive reaction.

6. It is suggested that susceptibility to *H. victoriae* and resistance to *P. coronata* depend upon comparable types of reactions induced by

toxins. The destruction of host tissue by toxins would facilitate growth of a facultative saprophyte such as *H. victoriae* while providing a physiological barrier to an obligate parasite such as *P. coronata*. The hypothesis of a gene with pleiotropic effects is in agreement with available genetical data.

IOWA STATE COLLEGE,
   AMES, IOWA

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ANNOUNCEMENT

With the current interest in the taxonomy and nomenclature of the fungi
a pressing need has arisen for certain basic works on the subject which have
long been out of print and of which even the few available copies are showing
signs of wear and tear.   Four outstanding items have been selected for re-
production by the photo-offset process provided sufficient advance sub-
scriptions are indicated.   The works in question together with the probable
price per volume or set follow.

> FRIES, E. *Systema Mycologicum.* The three volumes together with the
> *Elenchus Fungorum* and the index to the complete work ..... $ 50.00
> PERSOON, C. H. *Methodica Fungorum* 20.00
> LINDAU, G., and SYDOW, P. *Thesaurus Mycologicae et Licheno-*
> *logicae.* 5 volumes 90.00
> ZAHLBRUCKNER, A. *Catalogus Lichenum Universalis.* 10 volumes
> with index 175.00

The first and second of these are the classical works on which the
nomenclature of the fungi is based under the International Rules.   They
have not been available even as second-hand copies for many years.   The
third lists the literature of mycology and plant pathology up to 1910 and
is invaluable to all working on the historical aspects of any problem in these
fields.   The final item, which is to the lichenologist what Saccardo's *Sylloge
Fungorum* is to the Mycologist, is out of print and unobtainable.

In order that an idea of the number of prospective sales may be obtained,
it is requested that tentative subscriptions, either personal or institutional,
be sent to the undersigned.—JOHN A. STEVENSON, Bureau of Plant Industry,
Soils, and Agricultural Engineering, Beltsville, Maryland.

REPORT OF THE 40TH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The American Phytopathological Society held its fortieth annual meeting at the William Penn Hotel in Pittsburgh, Pennsylvania, on December 6, 7, and 8, 1948. The Northeastern Division met with the parent society. The North Central Division held an informal meeting. On the afternoon of December 8, a joint session was held with the Potato Association of America. Three hundred and eighty-seven members registered from forty states and six foreign countries. One hundred and twenty-three papers were presented in seventeen sessions and symposia. Conferences and symposia were held under the titles of ''The Teaching of Plant Pathology; Extension Workers' Conference; Nature and Methods of Inspection of Nursery Stock for Importation and Interstate Shipment; Stone Fruit Virus Disease Problems; and Fungicide Colloquium.''

The annual dinner held at the William Penn Hotel was attended by approximately three hundred. The entertainment at the dinner by the Westinghouse male quartet was received enthusiastically.

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THOMAS SPROSTON, JR.; M. F. KERNKAMP

Temporary Committee for 1949:

Publication of Special Material: DONALD CATION, Chm.; H. P. BARSS; GEORGE
KENT; W. H. TISDALE

Report of the Secretary. On December 3, 1948, the total membership was 1,497,
consisting of 143 life members, 1,211 regular members, and 143 applicants to be ac-
cepted at the Pittsburgh 1948 annual meeting. Nine members died during the year:
Cyril O. Bratley (life), Anthony Berg, Loran Blood, B. F. Lutman, William M. Scott,
George H. Smith, Albert F. Woods (charter, life), Sanford M. Zeller, and Howard S.
Fawcett.

The Society by mail vote of the members approved the proposal to affiliate with the
American Institute of Biological Sciences.

Report of the Treasurer. Statement of accounts for the year ending September 30,
1948.

Receipts:

Balance from 1947			$ 4,936.46
Annual dues:			
1947	$ 55.50		
1948	5,069.51		
1949–53	145.75	$5,270.76	
Sales		73.42	
Sustaining Associates		1,600.00	
Transferred from PHYTOPATHOLOGY		1.00	
			6,945.18
Total Receipts			$11,881.64

Expenditures:

Member subscriptions transferred to PHYTOPATHOLOGY
1947		$ 45.76	
1948		4,224.57	
1949–53		121.30	
			$4,391.63
Transferred to PHYTOPATHOLOGY for sales			73.42
			4,465.05
Office of Secretary			
Sec. work		90.30	
Stamps		74.15	
Printing		174.01	
Other		17.59	356.05
Office of Treasurer			
Sec. work		321.90	
Stamps		53.47	
Other		43.29	418.66
Office of President			
Printing			20.68
Biological Abstracts			100.00
Placement Committee			36.55
Sustaining Associate Committee			22.00
Annual Meeting			
Rooms		398.05	
Travel		159.81	557.86
Withholding Tax			24.00
Total Expenditures			$ 6,000.85
Balance on hand October 30, 1948			5,880.79
			$11,881.64

Report of the Business Manager. The costs of carrying out the functions of the Business Office increased this year over 1947 by $4,552.98. With 1418 members and 907 subscribers there was a total of 2356 copies of PHYTOPATHOLOGY distributed each month during 1948. This is 196 more than in 1947.

Statement of Accounts for the Year Ending September 30, 1948.

Receipts:

Balance on hand from 1947			$ 7,259.93
Subscriptions:			
1947	$ 156.55		
1948	5,646.50		
1949–50	800.30	$6,603.35	
Member subscriptions and sales transferred from APS			
1947	45.76		
1948	4,224.57		
1949–53	121.30		
Sales	73.42	4,465.05	
Sales back numbers of PHYTOPATHOLOGY		1,927.91	
Sales of Membership list		6.00	
Sales of 30-year index		99.15	
Reprints		1,552.28	
Excess illustrations		269.60	
Rockefeller Institute Contribution		600.00	
Interest on sinking funds		86.50	
Donations and Legacies fund		52.00	
Unused credit		55.95	
Excess abstracts		2.80	
Refunds		17.82	
Transfer to APS		1.00	
Advertising		1,917.19	
Total Receipts			17,656.60
Bank balance 1947 and receipts for 1948			24,916.53
Northwestern Federal Savings and Loan			5,340.54
Grand Total			$30,257.07

Expenditures:

Printing, distributing and storing PHYTOPATHOLOGY

Vol. 37, no.	9	$ 891.33	
	10	901.65	
	11	891.66	
	12	1,144.81	
Vol. 38, no.	1	1,005.52	
	2	843.49	
	3	1,201.56	
	4	987.10	
	5	1,073.30	
	6	1,051.90	
	7	1,146.91	
	8	1,028.18	
Postage		881.69	
Engravings		876.09	$13,925.19
Office of Business Manager			
Secretary		761.91	
Supplies		53.48	
Stamps		33.01	
Miscellaneous		159.08	1,007.48
Editor-in Chief			
Secretary		1,399.15	
Supplies		106.75	
Express		7.75	
Miscellaneous		9.39	1,523.04
Advertising Manager			
Secretary		72.00	
Postage		11.64	
Commission		135.67	
Miscellaneous		30.28	249.59
Purchase of back volumes			31.00
Refunds			116.47
Express			16.51
Printing			367.91
Miscellaneous			40.66
News Letter			87.70
Bank charges			1.03
Reprints			1,229.32
Transfer APS dues			1.00
Membership list			616.99

Total Expenditures		$19,213.89
Balance on hand:		
Checking account	5,702.64	
Northwestern Federal Savings and Loan	5,340.54	11,043.18

Grand Total	$30,257.07

Sinking Fund. The principal amount of the sinking fund remained the same as for the previous year.

First mortgage note, at 3.8 per cent interest, deposited with McLachlen Banking Corporation for collection	$ 500.00
U. S. Savings Bond, Series G, No. M19056026, 2½ per cent interest	1,000.00
Invested with the following:	
Jefferson Federal Savings & Loan Ass'n (accrued dividends, $103.65)	603.65
District Bldg. Loan Association (accrued dividends, $306.66)	1,806.66
National Permanent Bldg. Ass'n (accrued dividends, $517.68)	2,517.68
Northwestern Fed. Savings & Loan Association (certificates)	2,000.00
Perpetual Building Association (accrued dividends, $207.31)	1,207.31
Prudential Building Association (accrued dividends, $44.85)	251.19
Arlington and Fairfax Bldg. & Loan (accrued dividends, $109.80)	1,109.80

	$10,996.29
Less interest due PHYTOPATHOLOGY	1,289.95

	$ 9,706.34

Lyman Memorial Fund. This fund is obtained from voluntary contributions and now totals $3958.94. This amount is invested with the Brookland Building and Loan Association at 2½ per cent interest.

Balance on hand September 30, 1947	$ 3,807.96
Dividends December 31, 1947, and June 30, 1948	96.07
Voluntary contributions	54.91
	$ 3,958.94
Less interest due PHYTOPATHOLOGY	582.21
	$ 3,376.73

Additional Endowment:

War Savings Bonds, Series F (maturity value)	$ 1,125.00
War Savings Stamps (maturity value)	7.00
Total value at maturity	$ 1,132.00

Report of the Auditing Committee for the fiscal year, 1947–1948. We have examined the books of the Treasurer of the American Phytopathological Society and of the Business Manager of PHYTOPATHOLOGY for the period from September 30, 1947, to September 30, 1948. We found all receipts and expenditures and all funds of the Society and of PHYTOPATHOLOGY accurately recorded.

Signed: GUS A. MCLAUGHLIN
R. C. COOMBS

Report of the Advertising Manager.

	Number of Advertisements (Paid) Pages	Space Used Pages	Gross Income Dollars	Net Income Dollars
	83	67.5	$2,780.80	$2,457.82
Membership List	11	11	445.00	378.67
Total, 1948	94	78.5	$3,225.80	$2,836.49

Expenses: Stenographic and Miscellaneous—$112.84
Pages of non-paying ads:

Biological Abstracts: ½ page Jan., Feb., Oct., Nov.,	2
Placement Committee: ½ page Jan., July, Sept., Oct., Nov.,	2½
Phytopathological Classics: Apr., May, Sept., Oct., Nov.,	5
Forms for Bequest and Codicil: November	1
Total	10½

Report of the Editor-in-Chief. Volume 38 of PHYTOPATHOLOGY contains 1,040 pages in which 374 authors published 105 long articles, 38 phytopathological notes, 208 abstracts, and 6 book reviews. The report of the annual meeting of the Society was published in the April issue. Seven manuscripts have been withdrawn or rejected since December, 1947. Thirty manuscripts were in press on November 1, 1948, eleven were being revised by authors, and fifteen were being reviewed by the editors. Increased costs of publication have necessitated rigorous scrutiny of articles and numerous requests for condensation of manuscripts.

Report of the Editor, Phytopath News. As of December 1, three numbers of NEWS were published and distributed to members at a cost of $129.57. Another number awaits publication at an estimated cost of $43.26, thus making a total expenditure of approximately $172.83. If six numbers of NEWS are published in the same manner during 1949, a budget of $300.00 will probably suffice.

There are two alternatives for publishing PHYTOPATH NEWS: (1) continuation of the present system whereby NEWS is printed within a two-week period by a local press and is then sent to Lancaster, Pennsylvania for addressing and mailing. The chief advantages of this method are that no definite deadline must be met and that news items reach members with minimum delay. The principal disadvantage is the high cost of

printing and distributing each number.   (2) A page in PHYTOPATHOLOGY might be
designated for NEWS on a bimonthly basis or more frequently if it seems desirable.   The
chief advantage of this method would be a considerable saving in cost.   Disadvantages
include, a minimum period of six to eight weeks or more before news items would reach
members and additional lay-out work for the Editor-in-Chief of PHYTOPATHOLOGY.

A number of individuals representing fields of administration, research, extension, and
industry have expressed their interest in the new venture, and a few have contributed
suggestions for increasing the usefulness of NEWS.   The principal difficulty at present is
to obtain items for publication.   If everyone cooperates in supplying material, this
obstacle can be readily overcome.

**Report of Representative on Division of Biology and Agriculture, National Re-
search Council.**   The annual meeting of the Division of Biology and Agriculture, Na-
tional Research Council, was attended at Washington, D. C., May 6, 1948.   A full report
was sent to Council members on May 26, 1948.   During the year the American Institute
of Biological Sciences was launched under the sponsorship of the Division.   About 15
societies are now affiliated.   A permanent Executive Secretary has been appointed.

Representatives of the Division have participated in the representation of biologists
before the government committees concerned with selective service and war manpower.

The Committee on Genetics of Microorganisms is meeting in Washington in Novem-
ber to make future plans.

The Committee on Aerobiology, after necessary quiescence during the war period, is
being revivified under the chairmanship of Dr. E. C. Stakman.

**Report of Representative on the Governing Board of American Institute of Bi-
ological Sciences.**   The Society voted, by mail ballot, during the year to join the Ameri-
can Institute of Biological Societies as of January 1, 1949.   An organization meeting
was held at Washington on February 18, 1948; a second meeting was held in Washington
on September 13, 1948.   Both meetings were attended by Dr. Curtis May as observer.   Dr.
Milton O. Lee has been appointed as Executive Secretary.   Among the committees now
in action are one on Selective Service, and one on publications.

The Institute undertook to sponsor the Convention of Biological Societies in Washing-
ton in September, 1948, in which eleven societies participated.   The Governing Board has
instructed the Executive Secretary to work out suitable arrangements with AAAS for
1949 meetings in New York, and to inquire of constituent societies their wishes in regard
to meetings in 1950.

Report of Committee on Donations and Legacies.   The committee has placed a
total of $66.00 with the Treasurer and from this fund $24.00 has been used to provide
subscriptions for five foreign plant pathologists.   It is suggested that a new chairman
be appointed from the eastern area, preferably from near Washington, D. C., because of
facilities that are available there for securing needed information.

Report of Committee on Extension.   The Committee has sponsored an annual con-
ference of extension plant pathologists the last few years in conjunction with the annual
meeting of the American Phytopathological Society.   They also have a luncheon meeting
of the extension plant pathologists at which time mutual problems are discussed.   During
the past year the committee has been active on the following projects, which are under
supervision of selected personnel of the Extension Committee and act as subcommittees
of the Extension Committee: (1) Methods of filing and indexing bulletins, reprints, and
other subject matter materials by extension plant pathologists, (2) summary information
on airplane dusting and plant disease control in the United States, (3) the three-foot
book shelf for extension plant pathologists, and (4) subjects that would be included in the
graduate curriculum in training young extension plant pathologists.

Reports of these subcommittees will be presented at our annual conference in
Pittsburgh this year and summaries will probably be available for distribution later.

The following is an outline of the program of the extension conference at the
Pittsburgh meetings: (1) reports of various subcommittees, (2) present situation in ex-
tension plant pathology, (3) opportunities for making contributions to agriculture, (4)
progress in extension in varous states, and (5) an open discussion on future plans and
other pertinent subjects as requested by members attending.

Report of the Investment Committee.   Total investments of the Society for the
year ending September 30, 1948, are as follows:

Sinking Fund	$10,996.29
Lyman Memorial Fund	3,958.94
Additional Endowment:	
War Savings Bonds Series F (maturity value)	1,125.00
War Savings Stamps (maturity value)	7.00

The committee requests that Council and members approve the following proposals:

(a) That $11.50 from the general funds of the Society be added to the $7.00 in War Savings Stamps and a Series F War Savings Bond with a maturity value of $25.00 be purchased.

(b) That the War Savings Bonds having a total maturity value of $1,150.00 now carried as Additional Endowment be transferred to the Sinking Fund.

Report of the Editor, Phytopathological Classics. The year 1948 marked issuance of PHYTOPATHOLOGICAL CLASSIC No. 8: Berkeley's ''Observations, Botanical and Physiological, on the Potato Murrain,'' together with selections from his ''Vegetable Pathology.'' The selections were made by the Plant Pathology Committee of the British Mycological Society from articles Berkeley published in the GARDENER'S CHRONICLE AND AGRICULTURAL GAZETTE between the years 1854 and 1857. In this serialization, Berkeley outlined his extensive classification of plant diseases and detailed this with information then extant concerning the various causal fungi. The work, which originally consisted of 173 separate articles, is now brought together in No. 8.

Plans for No. 9 are under way, and the new CLASSIC will be ready for printing when the proceeds from sales of previous issues again make funds available. Under consideration for publication are: (1) Tozetti's Alimurgia, (2) M. Navashin's paper on *Sclerotinia betulae*, (3) selections from the works of Erwin F. Smith, with emphasis on the Smith-Fischer controversy, and (4) a review of recent Russian phytopathological research. Opinion has been solicited on the value of translating and reprinting the Alimurgia, the work in which Count Re sees historically the first real suggestion that plant diseases are caused by specific organisms. Both Dr. P. P. Pirone and Dr. J. C. Walker, who have reviewed the selection made by Professor Gabriele Goidanich, deem the work an interesting and worthwhile addition to PHYTOPATHOLOGICAL CLASSICS.

Suggestions concerning other likely papers for inclusion in the PHYTOPATHO-LOGICAL CLASSICS series are always welcome.

Report of the Business Manager, Phyopathological Classics.

Classic No. 1:	On hand, October 1, 1947 ... out of print		
Classic No. 2:	On hand, October 1, 1947	166	
	Sold during year	19	$ 9.50
	On hand, September 30, 1948	147	
Classic No. 3:	On hand, October 1, 1947	253	
	Sold during year	24	12.00
	On hand, September 30, 1948	229	
Classic No. 4:	On hand, October 1, 1947	317	
	Sold during year	22	16.50
	On hand, September 30, 1948	295	
Classic No. 5:	On hand, October 1, 1947	547	
	Sold during year	25	31.25
	On hand, September 30, 1948	522	
Classic No. 6:	On hand. October 1, 1947	633	
	Sold during year	25	18.75
	Gratis copy for use by editor	1	
	On hand, September 30, 1948	607	
Classic No. 7:	On hand, October 1, 1947	651	
	Sold during year	44	33.00
	Gratis copy for use by editor	1	
	On hand, September 30, 1948	606	
Classic No. 8:	On hand, March 1, 1948	1,006	
	Sold during year	174	$ 261.00
	Gratis copies sent out for advertising and to members of British Plant Pathology Comm. for aid in selecting M. J. Berkeley papers	11	
	On hand, September 30, 1948	821	

Value of books sent out (Fiscal Year 1947–48)	382.00
Money received during Fiscal Year 1947–48:	
For classics sent out	$ 344.50
On accounts due previous years	4.10
	$ 348.60
Total due on accounts	37.50

Assets:

Cash balance on hand, October 1, 1947	695.25
Receipts during Fiscal Year	348.60
	$1,043.85

Liabilities:

Postage for mailing out classics and for correspondence	$ 23.62
Expense of printing classic No. 8	715.50
Printing and cost of penny postcards for sales promotion	17.50
Bank service charge	1.10
Debit of account for returned check	1.50
	$ 759.22
Cash balance on September 30, 1948	$ 284.63
Total due on account September 30, 1948	$ 37.50

Report of the Placement Committee. During 1948, forty plant pathologists had applications filed with the Placement Committee. Prospective employers listed 53 positions with the Committee. A total of 192 individual applications were sent to employers. Incomplete reports indicate that at least four plant pathologists obtained positions through the efforts of this Committee.

Report of the Public Relations Committee. The Committee members published 17 popular articles on plant disease during the year. The procedure of dealing personally with editors of selected national and regional periodicals has been aided by assigning to each Committee member selected publicity outlets, on a subject-matter and geographic basis. It appears that each Committee member can accomplish more by limiting contributions to a few editors with whom he has developed effective personal contact, than by broader, impersonal activity. The Committee, as in the past, has met its expenses through remuneration for published articles.

Report of the Committee on International Cooperation. Following the effort of the Committee to help our foreign colleagues by sending copies of the 1946 Summary of Nationwide Tests with Newer Fungicides to leading plant pathologists in certain European countries, there was sent to the same addresses, in August, through the embassies or legations in Washington, a copy of the 1947 Summary (Plant Disease Reporter Supplement 176) with the compliments of the Committee.

Because of the very unsettled European situation, however, it seemed desirable to postpone until a more favorable time the project, previously proposed, to publish summaries of investigational results from various countries. During the coming year, however, it is hoped to publish such summaries from Mexico, Argentina, and possibly one or two other Latin-American countries.

Publication of a list of foreign plant pathologists, with special emphasis on those in Latin America, has been proposed. It is hoped that this can be published in CHRONICA BOTANICA and possibly in PHYTOPATHOLOGY. Attempt also is being made to furnish the Committee on Inter-American Scientific Publications (Harlow Shapley, chairman; Mrs. Christina Buochner, executive secretary) a list of some of the most important phytopathological publications in English that should be translated into Spanish. Reciprocally, some of the outstanding Spanish publications may be translated into English. In addition, because of the fact that relatively little plant pathological literature from the United States is available in many Latin American countries, the Committee on International Cooperation hopes to devise some way of stimulating the sending of such literature to outstanding phytopathologists or to libraries.

A few requests from other countries for literature references or other information were referred to our Committee during the year by various agencies, including UNESCO.

Report of Committee on Society Organization. The Committee on Society Organization has had no special problem for consideration this year, except a suggestion from the Secretary that it would be valuable to him to have the official names of the Divisions and a list of their officers for 1948. This information is given below:

Northeastern Division:

President	O. C. Boyd
Vice-President	D. H. Palmiter
Secretary-Treasurer	L. M. Black
Councilor	S. E. A. McCallan

North Central Division:
- President .... H. C. Young
- Vice-President .... J. C. Walker
- Secretary-Treasurer .... M. F. Kernkamp
- Councilor .... W. F. Buchholtz

Pacific Division:
- President .... G. W. Fischer
- Vice-President .... W. C. Snyder
- Secretary-Treasurer .... G. A. Zentmyer
- Councilor .... L. C. Cochran

Potomac Division:
- President .... W. J. Jeffers
- Vice-President .... C. L. Lefebvre
- Secretary-Treasurer .... J. B. Demaree
- Councilor .... Paul R. Miller

Southern Division:
- President .... I. L. Forbes
- Vice-President .... L. M. Blank
- Secretary-Treasurer .... J. A. Lyle
- Councilor .... S. G. Lehman

Report of the Committee on Plant Disease Prevention. Although the Committee as a unit has not made any concrete contribution, its members have been active. Several members have given considerable time to contacting key men in an attempt to imbue them with the danger of the introduction of new plant pathogens and the need for financial support. The military forces of the United States are fully aware of its existence. Two Committee members were called to Washington for consultation in regard to this matter.

The Committee is also sponsoring a conference at the annual meeting, to which representatives from the different states are invited. The representatives will be given opportunity to express themselves on the general nature of plant disease prevention, with emphasis on the following: (1) means of preventing the introduction of new pathogens or races; (2) methods of detecting new introductions; and (3) organization of plant disease information service, on certain epidemic diseases.

The following resolution was passed unanimously at the conference meeting of the Committee:

The Plant Disease Prevention Committee requests the President of the Society in consultation with the new chairman of this Committee to appoint a group of three to five members, to visit, at least, the Sub-Appropriation Committees of the two houses of Congress, the Budget Bureau, the Secretary of Agriculture, the Secretary of National Defense, and the Chiefs of Bureau of Entomology and Plant Quarantine and Bureau of Plant Industry, Soils, and Agricultural Engineering to present: (1) the urgent need for more effective protection of the major crops and trees against plant diseases, particularly plant diseases not now present in the United States, and (2) to prepare and present to each of the Administrations and groups visited an analytical statement of the critical problems and of the solution proposed.

Report of the Subcommittee on Regulatory Work and Foreign Plant Diseases. It was the understanding when this Committee was appointed that it was to consider problems on which its advice was asked by the federal plant quarantine service. During the year no requests were received.

Report of the Subcommittee on Seed and Plant Material Certification. Although the exact duty of this Committee is not too clear, I believe the members can render valuable service in an advising capacity and also as an aid in bringing together different agencies and organizations which have more or less common interest. Thus, the International Crop Improvement Association asked the chairman to present our viewpoint on diseases in relation to seed certification at their December meeting in Kansas City, Missouri. In addition, the Committee is sponsoring at the annual meeting of The American Phytopathological Society a symposium on ''The Nature and Methods of Inspection of Nursery Stock for Importation and Interstate Shipment.'' Topics for discussion include: (1) viewpoint of state pathologists, nurserymen, plant quarantine service (domestic and foreign), and research workers; (2) irregularities in inspection in different states in relation to interstate commerce and the need for minimum certification standards; and (3) problems involved in the identification of certain diseased nursery stocks.

Report of the Subcommittee on Utilization of Plant Pathologists and Field Facilities in National Emergencies. A report of progress of the Committee on the Utilization of Plant Pathologists and Field Facilities in National Emergencies follows:

1. The Committee has continued with its unfinished efforts of the previous year and has initiated a consideration of Dr. Waldee's suggestion on the utilization of plant pathologists in military government. No final action has been taken on those. It evaluated and approved a suggestion from Dr. Kent on the expansion of the committee's activities to include civilian defense. It sent, through the secretary, to one plant pathologist at each of the experiment stations its form letter of information about Research and Development Units for Reserve Officers. This letter called attention to the opportunity of plant pathologists who are reserve officers to obtain technical assignments in the armed forces through the authority of the Department of Army Circular 127, 1948. It also suggested that the information be brought to the attention of reserve officers at the state experiment stations and U. S. Department of Agriculture.

2. The following recommendations are presented to the Society:

(a) The American Phytopathological Society continues this Committee for 1949

(b) The American Phytopathological Society through some practical means as the "News Letter" inform its members in the United States that the Military Establishment has made provisions for qualified plant pathologists:

  (1) to obtain professional and technical commissions in the Officers Reserve Corps under authority of Department of Army Circular 210, dated July 14, 1948, and

  (2) if now a reserve officer, to transfer to technical assignments with appropriate promotion under authority of this circular.

(c) The American Phytopathological Society notify the American Institute of Biological Sciences (if and when affiliated with it) that the Committee on Utilization of Plant Pathologists and Field Facilities in National Emergencies exists and suggest that a similar committee be considered for appointment by that organization.

(d) The American Phytopathological Society offers its services (through the Committee on Utilization of Plant Pathologists and Field Facilities in National Emergencies) to the Secretary of National Defense for the purpose of:

  (1) studying jointly the offensive and defensive needs and potentialities (that security permits) of personnel trained in the science of plant pathology to the Army, Navy, and Air Force for National Defense, and

  (2) evaluating the military duties for which plant pathologists are qualified, and to assist in the drafting of job classification, description, and assignments.

Report of the Committee on Resolutions. The American Phytopathological Society expresses its real appreciation:

To W. C. Price, Chairman, and J. G. Leach, H. G. Guy, Allen Bauer, and O. E. Jennings, members of the committee on local arrangements, for their exacting efforts in assuring the success of the fortieth annual meeting;

To the management and staff of the William Penn Hotel for their considerate and courteous treatment of the Society and its members;

To the following sponsors whose assistance contributed so much to the excellence of the meeting facilities and the annual dinner: Carbide & Carbon Chemicals Corp.; The Dow Chemical Co.; E. I. DuPont de Nemours & Co., Grasselli Chemicals Department; The Harshaw Chemical Co.; Innis, Speiden & Co.; Koppers Co.; Monsanto Chemical Co.; Niagara Chemical Division, Food Machinery Corp.; Phelps Dodge Refining Corp.; Rohm & Haas Co., Agricultural & Sanitary Chemicals Division; Stauffer Chemical Co.; Sindar Corp.; S. W. Clark, Texas Gulf Sulphur Co.; Westinghouse Electric Corp.;

To the Westinghouse Quartet for furnishing the climax to a superb banquet; and

To the members of the Pittsburgh Convention Bureau for their generous supervision of registration.

The members of the Society express their thanks to President Kirby, Vice-President Valleau, and Secretary May for their efforts during the past year, and to Treasurer Richards for his services during the past two years.

Elections and Appointments. A committee of the Council opened and counted the ballots for president, vice-president, and councilor-at-large. W. D. Valleau, C. M. Tucker, and W. H. Tisdale were elected to these respective offices. The Council recommended and the Society approved the appointment of A. E. Dimond as Treasurer for a 3-year term, to succeed M. C. Richards at the close of the fiscal year.

The Council recommended and the Society approved the reappointment of Helen Hart as Editor-in-Chief of PHYTOPATHOLOGY for a 3-year term expiring in 1951. The Society thanks Dr. Hart for her meritorious services and untiring efforts in behalf of the Journal and the Society during the past three years.

The Council recommended and the Society approved the appointment of G. C. Kent as Editor for a 3-year term through 1951, and Harold T. Cook, Berch W. Henry, C. S. Holton, and J. Duain Moore as Associate Editors for 3-year terms, expiring in 1951. L. J. Alexander was reappointed Advertising Manager of PHYTOPATHOLOGY for 1949; L. C. Knorr was appointed Editor of PHYTOPATHOLOGICAL CLASSICS for 1949, and L. J. Tyler, Business Manager; J. C. Walker was appointed representative to the American Institute of Biological Sciences and remains as the Society representative to the Division of Biology and Agriculture of the National Research Council for 1949; R. S. Kirby was appointed as representative on the AAAS Council for 1949–50; Charles Chupp remains as representative on the Board of Editors, American Journal of Botany through 1950; H. H. McKinney was appointed Society representative to the American Type Culture Collection; and H. H. Thornberry was appointed as Society member of the Scientific Liaison and Advisory Board, Quartermaster Food and Container Institute for the Armed Forces.

Reports of Officers, Representatives, and Standing Committees are published on previous pages. Reports of Special and Temporary Committees are not published in the annual report. All committee reports submitted were considered and approved by the Council and accepted by the Society.

The Council recommended and the Society approved the following actions:

1. The Board of Editors is authorized to determine the disposition of the proposed lists of foreign plant pathologists now being considered by the Committee on International Cooperation;

2. continuation is authorized of publication of the NEWS LETTER, and K. W. Kreitlow is reappointed Editor. The unused balance of funds appropriated for 1948 are made available for use in 1949 and an additional sum of three hundred dollars is also made available for the NEWS LETTER in 1949;

3. the Society shall meet with the AAAS in New York City in 1949;

4. the Program Committee is authorized to develop a tentative schedule of meeting for 1950 and 1951. It is suggested that in 1950 the meetings be held either in the South or in the Great Plains and that in 1951 an effort be made to meet with the Entomological Societies, which it is understood now plan to meet in 1951 in the area between Chicago and Pittsburgh. The date of the meetings shall be determined by the Program Committee with preference for early December or early February;

5. it is recommended that the Constitution be amended in prescribed manner to provide for a new class of members to be known known as Emeritus members. It is proposed that the qualifications for Emeritus membership are as follows: (a) any member of the Society, who has been a member for 20 years, may upon retirement from professional duties apply for Emeritus status; (b) an Emeritus member shall retain all rights and privileges as a member of the Society but shall not receive PHYTOPATHOLOGY unless he so desires, in which case he shall receive it at cost;

6. the committee on Society Organization is dismissed with the thanks of the Society. This Standing Committee is discontinued;

7. the Society commends the Membership List Committee for their careful work and the excellence of the List which was published as section 2 of the August 1948 issue of PHYTOPATHOLOGY. The Society thanks S. E. A. McCallan for the preparation of the map showing the geographical distribution of members of the Society. The Membership List Committee is discharged with the sincere thanks and gratitude of the Society.

8. Article 4 of the Standing Rules shall be amended to read as follows: the Secretary, the Treasurer, the President or in his place the Vice-President, are authorized to receive reimbursement from the Society for travel expenses in connection with their attendance at the annual meeting. In addition the Editor-in-chief of PHYTOPATHOLOGY is also authorized to receive reimbursement for travel expenses to the annual meeting.

9. In accordance with previous Society action when affiliation with the American Institute of Biological Sciences was approved, Article I(a) of the Standing Rules shall be changed to read as follows:

(a) The dues for all annual members as defined in Section 2(b), Article II of the Constitution, including subscription to PHYTOPATHOLOGY, shall be $7.00 a year, payable by February 1. The Business Manager is authorized to discontinue sending the Journal to members whose dues have not been paid by this date.

10. The Treasurer is authorized to pay $850.00 to the American Institute of Biological Sciences as the membership fee for the Society for 1949.

11. In accord with common procedure, the Treasurer is authorized to pay to the new Treasurer and Business Manager of PHYTOPATHOLOGY necessary travel expenses incurred incident to assuming his new duties.

12. A. J. Riker is delegated to act with the President, Business Manager of PHYTOPATHOLOGY, ~~and the~~ Editor-in-chief with respect to publication problems of PHYTOPATHOLOGY.

The following resolution was introduced from the floor and after discussion was approved.

WHEREAS: The Plant Disease Reporter, issued by the Division of Mycology and Disease Survey, has proved to be invaluable in advancing plant disease research and extension work in this country and

WHEREAS it is believed by many that its full value as a reference source has not been realized owing to the somewhat bulky and fragile form imposed by the mimeograph process now in use and

WHEREAS if either a photo offset or a printing process could be employed, using a suitable format, its value for reference and research would be improved

BE IT RESOLVED THEREFORE that The American Phytopathological Society urge that the Plant Disease Reporter be published in more permanent form for reference purposes, if this can be done without sacrificing promptness of issue which has contributed so much to its usefulness

BE IT FURTHER RESOLVED that copies of this resolution be sent to the Secretary of Agriculture, the Administrator of the Agricultural Research Administration, the Chief of the Bureau of Plant Industry, Soils, and Agricultural Engineering, and the Head of the Division of Mycology and Disease Survey.

<div style="text-align:right">Curtis May, Secretary</div>

MINUTES OF THE BUSINESS MEETING, NORTHEASTERN DIVISION, THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, DECEMBER 6, 1948

President O. C. Boyd presided. The minutes of the business meeting of July 23, a brief account of the summer meeting at Ithaca and Geneva, New York, and the Treasurer's report were read by the Secretary-Treasurer, and approved by the membership. Dr. James G. Horsfall, chairman of the Committee on Nominations, placed the following names before the members: President, D. H. Palmiter; Vice-President, E. M. Stoddard; Secretary-Treasurer, L. M. Black; and Councilor, S. E. A. McCallan. No further nominations coming from the floor, it was moved, seconded, and carried that the Secretary record a unanimous ballot for the above candidates. Discussion regarding the time and place of the next meeting revealed that a majority favored a summer meeting without papers. . A motion to leave further details of the summer meeting to the Executive Committee was passed. Dr. D. H. Palmiter proposed that "Inasmuch as the officers of the parent society made all arrangements for the joint meeting of the Northeastern Division with the parent society: be it resolved that the Northeastern Division express its thanks to the President, Secretary, and other officers of The American Phytopathological Society." The resolution was adopted and the Secretary instructed to forward copies to President Kirby and Secretary May.

<div style="text-align:right">L. M. Black, Secretary-Treasurer</div>

CHLAMYDOSPORE GERMINATION, NUCLEAR CYCLE, AND ARTIFICIAL CULTURE OF *UROCYSTIS AGROPYRI* ON RED TOP

M . J . T H I R U M A L A C H A R A N D J A M E S G . D I C K S O N

The flag smut of grasses incited by *Urocystis agropyri* (Preuss.) Schroet. occurs on a wide range of grass hosts, several of which are of economic importance. Clinton (3) in North American Flora reported it on *Agropyron repens* (L.) Beauv., *A. spicatum* (Pursh) Scribn. and Smith, *A. smithii* Rydb., *Bromus ciliatus* L., *Calamagrostis canadensis* (Michx.) Beauv., *Elymus arenarius* L., *E. canadensis* L., *E. canadensis* var. *robustus* Mackenz. and Bush, and *E. virginicus* L. Davis (4) collected the smut near Madison, Wisconsin, on red top (*Agrostis alba* L.). Zundel and Barnhart (19) record a more extensive host range for the smut in the United States including *Poa pratensis* L., *Hordeum nodosum* L., and others. Recently more interest has been aroused in the *Urocystis* group on grasses by the findings of Fischer and Holton (7) that the flag smut of wheat (*U. tritici* Koern.) infects certain grass hosts. Since *U. tritici* and *U. agropyri* are similar and morphologically indistinct, they concluded that the two belong to the same species and so reduced *U. tritici* to a synonym of *U. agropyri*.

The life cycles of *Urocystis occulta* (Wallr.) Rab. on rye and *U. tritici* on wheat have been investigated in some detail (15, 13). In so far as the writers can determine, the chlamydospores of *U. agropyri* from grass hosts have not been observed germinating. The cross inoculations carried out by Fischer and Holton (7) were done mostly by using spores from the flag smut of wheat and, in a few cases, with spores from grass hosts. The cross inoculations were made by submerging the seed under partial vacuum in aqueous suspension of the smut spores. The appearance of symptoms on the developing plants was taken as indicating successful infections.

In the course of investigations of the stripe smuts of the grasses, the chlamydospores of *U. agropyri* from red top were germinated and the fungus was cultured on malt-extract media. The present paper is a preliminary report of the cultural types and cytology of the fungus from red top.

SYMPTOMS

The external symptoms produced by the flag smut on red top are similar to those of *Ustilago striiformis* (Westend.) Niessl var. *agrostidis* occurring on the same host. Both smuts occur on *Poa pratensis*, *Elymus canadensis* and *Agropyron repens* in Wisconsin, occasionally on the same plant. In the field the two can be differentiated fairly reliably by the more brownish color of the sori in *U. agropyri* and its earlier appearance in the spring.

333

In several bluegrass pastures near Madison, Wisconsin, the writers have noticed as high as 25 per cent flag smut infection in early spring before there was any manifestation of *Ustilago striiformis*.

Small striae, 0.5 to 1.0 mm. long, appear first, which in due course develop into elongated stripes, frequently extending the entire length of the leaves. The sori rupture the epidermis and dusty masses of spores appear on the surface of the pustules, following which the leaves become shredded longitudinally. In the newly infected plants, the leaves manifest a shredded condition and reduced culm elongation but do not show other malformations. On the other hand, the shoots regenerating from culms of plants smutted in the preceding season develop thin, narrow leaves clustered around the condensed internodes giving a witch's broom effect (Fig. 1, A). Many of the diseased plants dry out during midsummer and fail to recover following the advent of favorable conditions. The smutted plants rarely produce inflorescences.

MATERIALS AND METHODS

The fungus was studied from red top only, collected in the vicinity of Madison, Wisconsin. The smutted leaves were fixed in Karpechenko's modification of Nawaschin's fluid and formalin acetic alcohol. Sections 8–10 μ thick were cut and stained with Heidenhain's iron-alum haematoxylin with orange G as counterstain or with Newton's iodine, gentian violet. The chlamydospores were germinated and stained by the method described for rust teliospores by Thirumalachar (16). The germinating spores were transferred aseptically onto potato-dextrose agar or malt agar to secure the cultures of the fungus.

CHLAMYDOSPORE GERMINATION

As stated earlier the chlamydospores of *Urocystis agropyri* from the grasses have not been germinated previously. Schellenberg (14), Ciferri (2), Liro (11), Fischer and Hirschhorn (6), and others have reported failure. Recently Kreitlow (10), studying the smut on timothy, failed to secure germination in spite of using treatments to break the dormancy. Thirumalachar and Dickson (18) reported the first successful germination of the chlamydospores from red top and noted some of the cultural characters in vitro. The chlamydospores were fixed to the slides by wetting and drying and the slides were inverted over a water surface as described earlier. In the early experiments several of the spores developed promycelia with a whorl of sporidia at the apex after 45 days incubation at about 24° C. Pretreatment with benzaldehyde (3:2,000,000) as used by Stakman *et al.* (15) for breaking the dormancy of chlamydospores of *U. occulta* and the addition of fragments of green host tissue found useful by Noble (12) to stimulate the germination of *U. tritici* were not effective in increasing germination percentages. Satisfactory germination was obtained when the spores were fixed to the slides by repeated wetting and drying, then placed

in Coplin jars containing distilled water and incubated at 40° C. for eight hours. After this treatment the slides were removed and inverted over the water surface in the usual manner; several of the spores had germinated after 5 days. Long promycelia with a whorl of three to four sporidia characteristic of the Tilletiaceae developed from the germinating spores (Fig. 2, b to d).

<center>CYTOLOGY OF THE FUNGUS</center>

Stained material of various stages of spore germination showed the nuclear behavior typical of the smut fungi. The fusion nucleus migrated into the promycelium (Fig. 2, b) and there divided twice to form four haploid nuclei. After the nuclear divisions, three to four sporidia developed at the apex of the promycelium and a single haploid nucleus migrated into each sporidium (Fig. 2, c to e). In instances where three sporidia were formed, the supernumerary nucleus either remained within the promycelium or infrequently migrated into one sporidium (Fig. 2, f) to initiate the dicaryophase. After sporidial formation, conjugation tubes were formed between the compatible pairs of sporidia while still attached (Fig. 2, d to f). After sporidial conjugation the dicaryotic nuclei migrated into the infection hypha produced, usually from the conjugation tube. The infection hyphae elongated rapidly with a tendency to become aerial (Fig. 1, B). In many instances the promycelia developed two sporidium-like structures which elongated rapidly into slender hyphae (Fig. 2, g). These were usually binucleate which indicated direct migration of paired nuclei or the initiation of the dicaryophase directly without sporidial conjugation. This type of dicaryophase initiation has been described for *Urocystis occulta* by Stakman *et al.* (15) and for *Cintractia montagnei* by Rawitscher (13). When the germinating spores were submerged in water, the promycelium elongated indefinitely, septa developed at the base, and the protoplasmic contents migrated towards the apex.

Chlamydospore development within the host was studied in microtome sections of the smutted leaves. The mycelium and spore formation were similar to those described by Stakman *et al.* in *Urocystis occulta*. The spores formed from the dicaryotic mycelium which concentrated in a closely packed mass replacing the mesophyll cells between the vascular bundles in the leaf. The fertile cells with dense cytoplasm were surrounded by vacuolate hyphal cells, with nuclei in various stages of degeneration, that later constituted the sterile cells of the spore ball (Fig. 2, a). The mature spores were composed of one to three fertile cells surrounded completely by hyaline to pale cinnamon yellow sterile cells. The fertile spores were thick-walled and reddish-brown. Nuclear fusion occurred in the fertile cells in the early stages of spore formation.

<center>ARTIFICIAL CULTURE OF THE SMUT</center>

Cultures of *Urocystis agropyri* were obtained by transferring germinating chlamydospores aseptically onto potato-dextrose agar or malt agar.
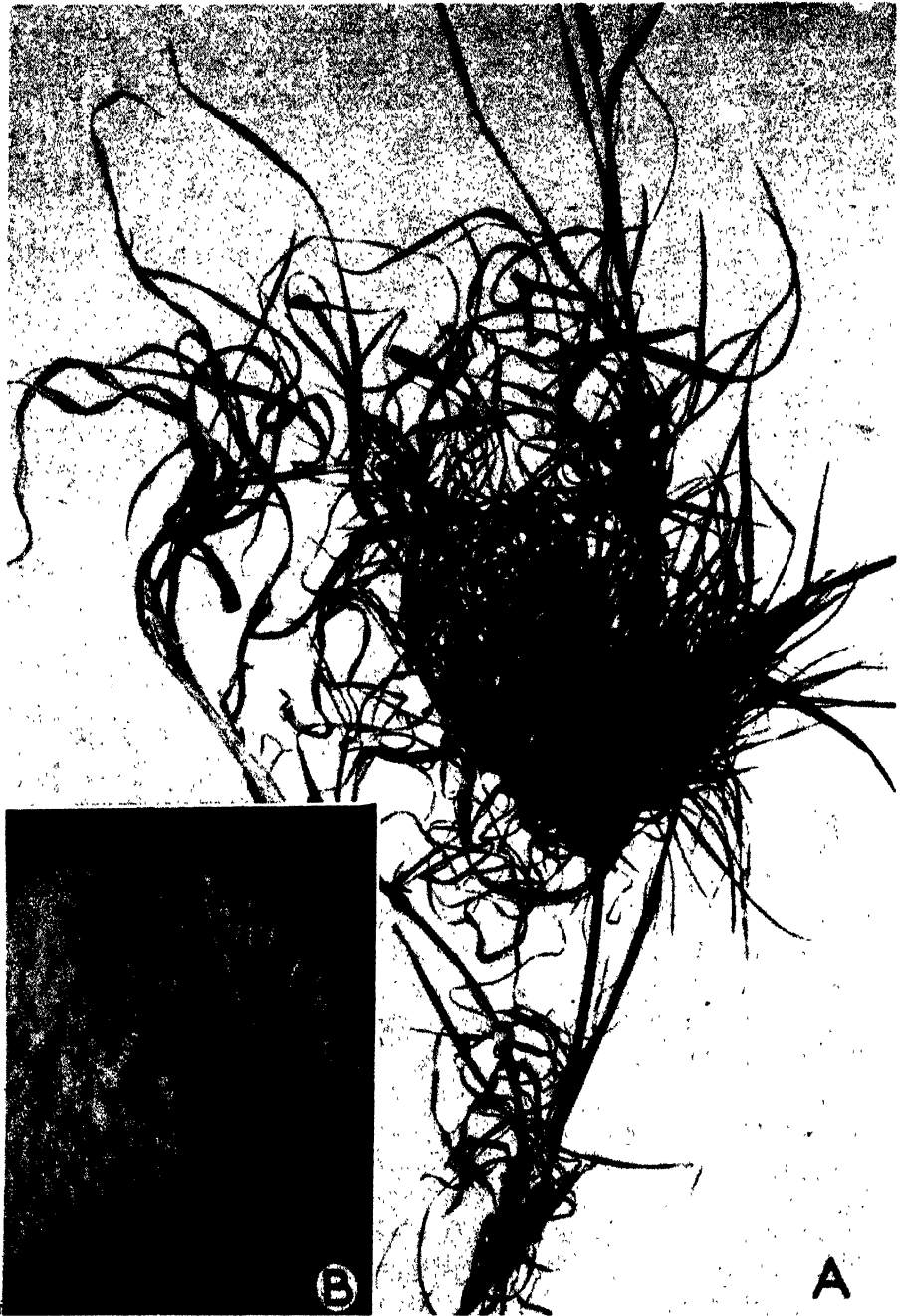
Fig. 1. *Urocystis agropyri* on red top.
  A. Witch's-broom type of growth from culms of smutted older plants. Natural size.
  B. Mycelial culture on malt agar. Natural size.

In other instances, the germinating spores were distributed on water agar and the single spores were picked out by Keitt's cutter and transferred to nutrient media. Lacking well developed aerial mycelium, the developing colonies were smooth, glistening, and flat. At temperatures between 20°
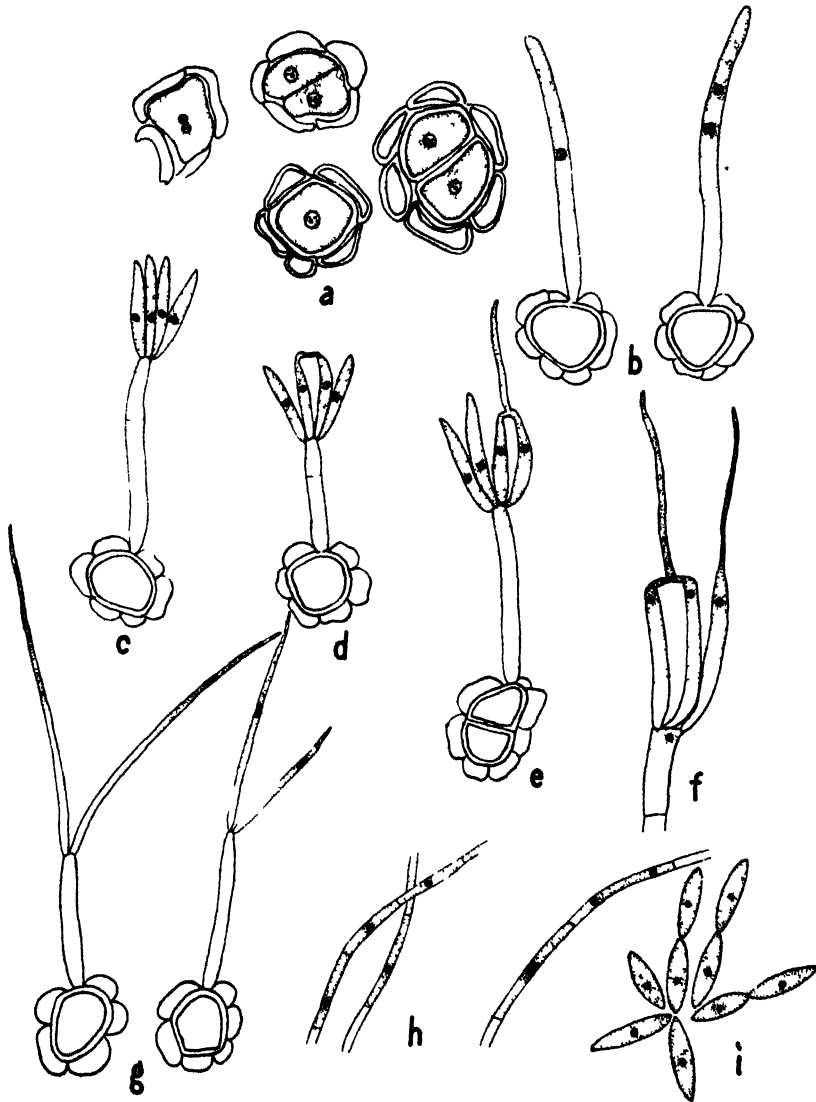
FIG. 2. Chlamydospore development, spore germination, and mycelial variants in *Urocystis agropyri*. (Camera-lucida drawings ×600 except f ×1200.) a. Young and mature sporeballs. b. Early stages of spore germination. c. Promycelium with four sporidia. d, e, and f. Conjugation of sporidia. g. Direct migration of the paired nuclei into branches. h. Dicaryotic and haploid hyphal cells. i. Sporidia from sectors in mycelial cultures.

and 24° C. a counter-clockwise spiral contour was pronounced on the colony surface (Fig. 1, B).

In the mycelium developed in culture, the young hyphae were multinucleate but showed the pairing of the dicaryotic nuclei. Septa were formed soon resulting in the characteristic dicaryotic mycelium. In several of the sectors evident in culture, a large number of uninucleate hyphae were found associated with the dicaryotic mycelium (Fig. 2, h). In some of these subcultures the stages in the separation of the paired nuclei and subsequent formation of uninucleate cells were observed. Such dediploidization of the dicaryotic hyphae has been described in several other smuts such as *Ustilago longissima* (1), *U. hordei* and *U. kolleri* (5), *U. zeae* (8), and *U. striiformis* var. *agrostidis* (Thirumalachar and Dickson, in press).

In some instances small glistening white colonies with convoluted surface were produced as sectors in the cultures of the dicaryotic hyphae which on examination proved to be sporidial in nature. The sporidia were ovate-ellipsoid, hyaline, and thin-walled (Fig. 2, i). These colonies developed rapidly by yeast-like budding characteristic of smut sporidia. They resembled very closely the sporidial colonies obtained in the subcultures of the dicaryotic mycelia of *Ustilago striiformis* var. *agrostidis*. No conjugations between sporidia were obtained. However, at temperatures between 20° and 24° C. several mycelial sectors reappeared in these sporidial colonies.

DISCUSSION

The morphology of spore germination and the nuclear cycle in *Urocystis agropyri* on red top was similar to the general type recorded for other species of *Urocystis*. The mycelium in the host was dicaryotic and the nuclear fusion occurred within the young chlamydospore. Assuming from the cytological evidence that the reduction division occurred within the promycelium, the dicaryophase was initiated again by the conjugation of sporidia or by the direct migration of two haploid nuclei into the slender branches developed under some types of germination.

The development of the mycelium and sporidia on media was similar to that of *Ustilago striiformis* var. *agrostidis* occurring on the same host. There was a marked parallelism between the two species in mycelial development and in the production of sporidial sectors by dediploidization. Typical chlamydospores of *U. agropyri* have not been observed in culture although spore-ball-like structures without differentiated fertile and sterile cells were present in old cultures of the dicaryotic mycelium. Thick-walled, resting cells occurred frequently in old cultures. Kniep (9) reported the formation of true chlamydospores in *U. anemones* in culture or the development of the fungus from chlamydospore to chlamydospore in vitro.

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THE PYCNIDIAL STAGE OF THE WALNUT BRANCH WILT FUNGUS, EXOSPORINA FAWCETTI

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In 1947 the writer (7) proposed the name *Exosporina fawcetti* sp. nov. for a fungus causing branch wilt in Persian walnut trees (*Juglans regia* L.). This fungus, also found by the writer on branches of lemon (*Citrus limonia* Osb.), California black walnut (*Juglans hindsii* Jep.), and European chestnut (*Castanea sativa* Mill.), proved to be identical with one reported by Fawcett (3) from California on frost-injured branches of orange (*Citrus sinensis* Osb.) and grapefruit (*C. grandis* Osb.). Fawcett (3) noted the similarity between his fungus and a fungus described as *Torula dimidiata* by Penzig (6) in 1887. Recently Calavan and Wallace (2) proved isolates of *E. fawcetti* from citrus to be pathogenic to grapefruit trees.

The generic classification of *Exosporina fawcetti* was based on its production of brown, single-celled, irregularly shaped conidia (arthrospores) by segmentation of numerous parallel, closely packed hyphae, which arose from a slight hypostroma beneath the bark periderm. Such a fruiting structure was regarded as a sporodochium. On cornmeal agar the fungus produced an elaborate multicellular hypostroma which bore the arthrospores on its surface. In one series of cultures, one to two locules developed in this stroma-like body (Fig. 1, A). The locules were filled with structures resembling pycnospores, but as these never matured they were not identified with certainty. Subsequent attempts to produce a pycnidial stage in artificial media and on steam-sterilized walnut twigs failed.

On another occasion, stromata with pycnidial locules were found scattered among the arthrospores of *Exosporina fawcetti* on one small walnut twig. Since all but a few of the pycnospores had been discharged none was obtained for culturing. Extensive search for two years failed to reveal another case of a similar nature.

The writer recognized the similarities between his fungus and the toruloid stage of *Hendersonula toruloidea*, which was described by Nattrass (4) in 1933, but he did not feel justified in reporting it under that name.

In December, 1947, at Davis, California, numerous black carbonaceous pycnidial stromata were found on cankers developed by inoculating the trunks of young Persian walnut trees with *Exosporina fawcetti* (Los Molinos isolate) in May, 1947 (Fig. 1, C). A few arthrospores typical of those of *E. fawcetti* were clustered over and around the stromata.

In September, 1948, pycnidia and pycnospores identical with those mentioned above were found on pieces of walnut twigs that had been surface-sterilized with mercuric bichloride, placed in culture tubes containing a small amount of water, and inoculated with *Exosporina fawcetti* on Novem-

ber 19, 1947.   The tubes were kept in the laboratory near a north window and
sterile water was added occasionally.
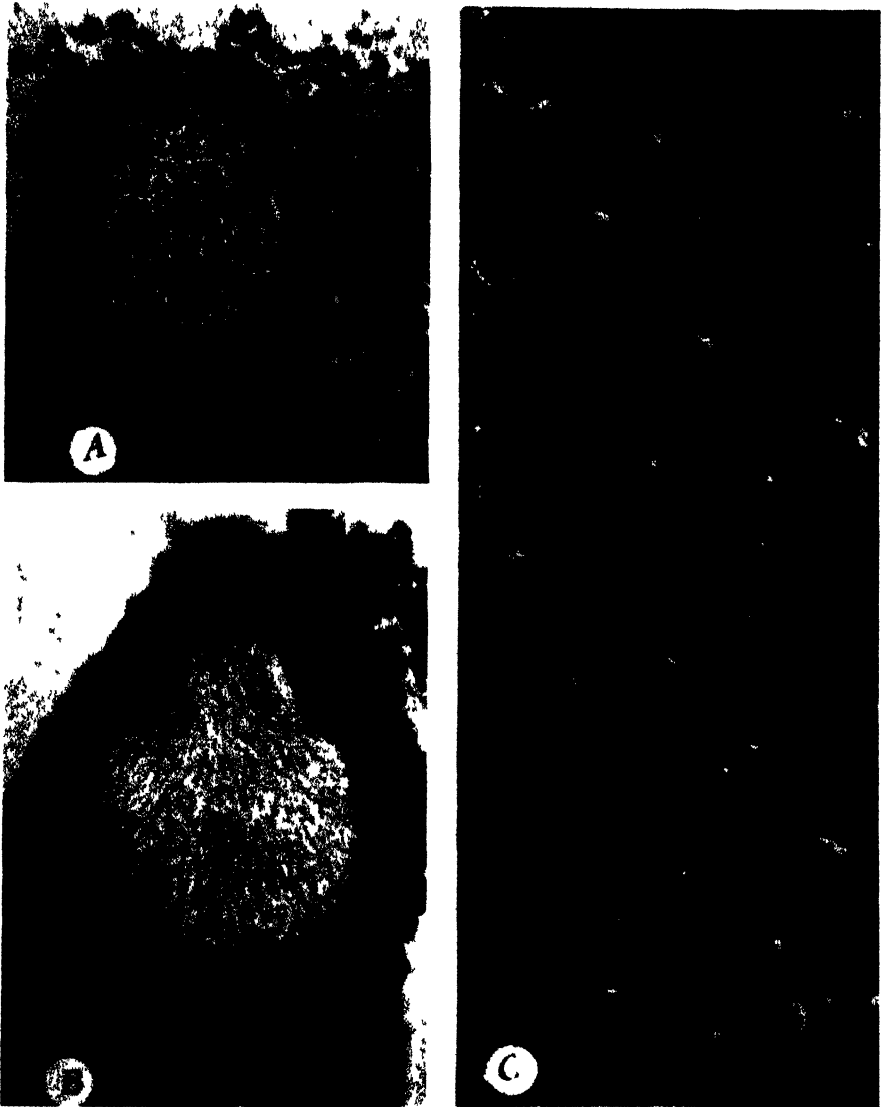
FIG. 1.   A. Locule in a stroma like structure produced by *Exosporina fawcetti* on
cornmeal agar and filled with what appear to be pycnospores.   The free arthrospores at
top of photograph were produced on the surface of the stroma-like structure.   B. Single
pycnidium on a basal stroma which developed beneath the periderm of a walnut twig
inoculated with *E. fawcetti*.   C. Stromata breaking through the periderm of a walnut
twig inoculated with *E. fawcetti*.

DESCRIPTION OF THE PYCNIDIAL STAGE

One to seven black pycnidia were partially embedded in each stroma.
The necks of the pycnidia were separate and arose to a height of 50 to 70μ

(Fig. 1, B, Fig. 2, D). Stromata varied between about 125 to 500μ in diameter and 150 to 225μ high. The flask-shaped pycnidial locules (Fig. 1, B), measuring 75 to 100μ in diameter and 100 to 150μ long and occurring at different levels in the stroma, were without walls differentiated from the rest of the tissue.

The non-septate, hyaline to slightly yellow, elongate, guttulate pycnospores (Fig. 2, A) were borne on slender, flexuous sporophores measuring 3 to 4μ broad and 10 to 15μ long. The spores measured 3.2 to 6.5μ wide (average, 5.3μ) and 11.9 to 16.0μ long (average, 14.6μ).

When the bark was wetted, pycnospores were extruded from the pycnidia in white cirri (Fig. 2, D). Within 24 hours at room temperature (23°–25° C.) the cirri became somewhat darkened and after 48 to 72 hours, jet black. The content of many of the spores from partially discolored cirri
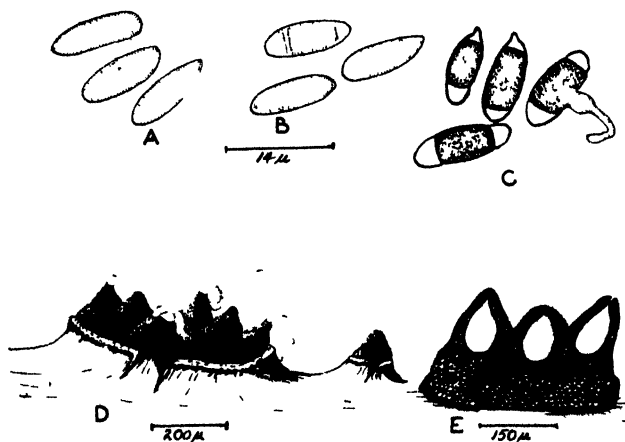
FIG. 2. Pycnidia and pycnospores of *Exosporina fawcetti*. A. Non septate hyaline pycnospores from pycnidium. B. Septate pycnospores about 48 hours after extrusions from the pycnidium. The spores at this time are sub hyaline. C. Pycnospores about 72 hours after extrusion from pycnidium. The center cell is darker than the terminal cells and its content is rounded at the ends, causing the cross walls to bulge into the end-cells. D. Habit sketch of two stromata, one with six pycnidia, the other with one. Pycnospores are extruded in white cirri which soon turn black. E. Vertical section through a stroma with three pycnidial locules.

were separated into three parts (Fig 2, B), but under the ordinary microscope no walls could be distinguished at these separations. Under cross Nicol prisms of the polarizing microscope, however, these areas of separation exhibited the anisotropic or double refraction phenomenon, indicating that cellulose was present.

Most pycnospores from jet black cirri were two-septate, with thicker walls than non-septate spores (Fig. 2, C). The contents of the center cells, which were darker in color than those of the terminal cells, were rounded at the ends. Consequently, the septa, which could be detected under crossed Nicol prisms, bulged into the end-cells. In many cases the spore wall around the terminal cells was noticeably wrinkled and sometimes collapsed inward.

Although the hyaline, non-septate pycnospores germinated readily in

double-distilled water at 25° C., only a very few of the three-celled colored spores germinated under identical conditions. Later it was found that from 60 to 70 per cent of the colored spores germinated when placed in water drops on the surface of potato-dextrose agar.

CULTURAL FEATURES

Single hyaline, non-septate pycnospores were isolated and placed on potato-dextrose agar. After incubating about a week at 25° C., mycelium extended across the plate, and a pulverulent surface growth, which was first white then greenish-black to black, developed. This surface growth was composed of numerous branched, aerial hyphae which become septate throughout their length, producing dark brown, irregularly spherical, ovoid, or wedge-shaped segments. The entire structure broke apart into single-celled segments (arthrospores) when placed in water. These germinated readily on water agar at 25° C.

On potato-dextrose agar the fungus was characteristic for *Exosporina fawcetti*. The arthrospores which developed from single pycnospores averaged 4.6 μ and 7.3 μ long, the mean dimension being 5.9 μ. This is slightly, but probably not significantly, larger than the mean dimensions of 5.1 to 5.5 μ obtained in measuring the arthrospores of various isolates of *E. fawcetti*.

The same fungus grew out of chips of wood removed aseptically from beneath the cankers on which the pycnospores were produced. Within two or three weeks at room temperature, sporodochia ½ to 1 mm. in diameter and bearing numerous arthrospores on the surface developed on chips of wood planted on potato-dextrose agar. No evidence of a pycnidial stage, however, was found in such cultures grown for several months.

EFFECT OF TEMPERATURE ON GERMINATION OF SPORES

According to earlier tests (7), optimum germination of the arthrospores and growth of the mycelium of *Exosporina fawcetti* occurs between about 30° and 33° C., whereas these processes are much reduced at 16° C. or below. Judging from the data in table 1, the optimum germination of non-septate pycnospores from the host and arthrospores from artificial media also occurs at 30° C. or more.

TABLE 1.—*The germination of pycnospores and arthrospores of Exosporina fawcetti at various temperatures*

Temperature in degrees centigrade	Percentage of spores germinating after 23 hours in water drops	
	Pycnospores	Arthrospores
6	0	0
9	14	0
15	76	44
21	80	46
25	87	60
30	98	73

PATHOGENICITY TO PERSIAN WALNUT, PEACH, AND APRICOT TREES

In June, 1948, the Los Molinos isolate of *Exosporina fawcetti* and an isolate obtained from a pycnospore were inoculated in branches of the Franquette variety of walnut, the Tilton variety of apricot, and the Phillips Cling variety of peach. Pieces of agar bearing mycelium and arthrospores of the respective isolates were inserted into cuts made in the sapwood. Waxed paper was placed over the cuts and bound with adhesive tape.

Within three weeks the two isolates produced similar dark brown to black necrotic areas 2 to 3 inches long in the bark and sapwood of the three hosts. Gum exuded profusely from inoculated cuts in apricot and peach branches. The toruloid conidial growth, which was recovered in isolations from cankers produced by each isolate, proved identical in all respects.

COMPARISON OF THE FUNGUS WITH HENDERSONULA TORULOIDEA NATTRASS

In 1933 Nattrass (4) reported isolating *Hendersonula toruloidea* from apples, peaches, and apricots suffering from a die-back in Egypt. This fungus was not considered the primary cause of the die-back and no inoculations were made to test its pathogenicity. It will be seen from the following review of Nattrass' description that the California fungus closely resembles *H. toruloidea* in morphological and cultural features.

On artificial media, for example, *Hendersonula toruloidea* produced at first a white pulverulent aerial growth which eventually became olivaceous black. Short, branched, convolute hyphae rising from the agar segmented into chains of "thallospores" (arthrospores). When mature, the thallospore segments were citrine drab to bone brown in color, globose, ovoid, or subcuneiform in shape, sometimes 1-septate, $4\,\mu$ broad and 4 to $10\,\mu$ long. Optimum temperature for growth of the fungus was 30° to 35° C.

This, the "Torula" stage, was the only one Nattrass (4) found on artificial media, but on inoculated cotton and willow twigs the fungus produced black carbonaceous pycnidia, single or in small aggregates, on a subicle or rudimentary stroma.

Two years after first isolating the fungus Nattrass (4) found its pycnidial stage in nature. One or more pycnidia immersed in a stroma became erumpent through the bark periderm. Pycnidial locules, which occurred at different levels in the stroma, were not surrounded by a wall differing from the stromatic tissue.

When wetted the pycnidia extruded white cirri which contained oblong, single-celled, hyaline to slightly greenish pycnospores, 4–$5\,\mu$ wide and 12-$13.5\,\mu$ long. After a time the cirri turned jet black and the pycnospores became two-septate. The center cells of such spores were dark brown, the terminal cells subhyaline. These spores were said to germinate less readily than hyaline spores.

In nature the arthrospores were borne in chains on a basal plate of fungus tissue. Though Nattrass (4) did not follow the development of

these plates, he believed they later enlarged into stromata which produced pycnidia.

Dr. E. W. Mason, Mycologist of the Commonwealth Mycological Institute, Kew, England, kindly sent the writer a specimen of *Hendersonula toruloidea* which Natrass deposited at that institution in 1930. The material was said by Dr. Mason to be a culture of the fungus on twigs.

Both arthrospores and pycnidia were present on this material. The former, though somewhat more regularly sphaeroid or ovoid than those of the California fungus, were about the same size (4.9 to 6.9μ), average mean diameter 5.6μ. Pycnospores from the pycnidia were hyaline, guttulate, elongate, 3.3 to 4.3μ broad (average 3.9μ) and 9.9 to 13.6μ long (average 11.6). No dark three-celled spores were found. The one- or two-loculate stromata were very similar in shape to those produced by the California fungus (Fig. 1, B; Fig. 2, D and E).

Other reports of Hendersonula toruloidea. Nattrass (5) later found *Hendersonula toruloidea* on citrus trees in Cyprus. Fawcett (3, p. 218 and 222) reports that in California A. J. Olson isolated a fungus resembling *H. toruloides* from frost-injured citrus branches following a freeze in 1931. Upon inoculation, the fungus produced lesions in citrus branches and black masses of hyphae and spores beneath the periderm.

In 1937 Bates (1) reported isolations of *Hendersonula toruloidea* from orange fruit in southern Rhodesia. On such fruit it produced a dark brown pliable decay. Fawcett (3, p. 464) also obtained a decay of oranges and lemons inoculated with the fungus.

CONCLUSIONS AND SUMMARY

In December, 1947, at Davis, California, pycnidial stromata developed beneath the bark of young Persian walnut trees inoculated some months earlier with *Exosporina fawcetti*, the pathogen of walnut branch wilt. Isolates from pycnospores produced a toruloid conidial (arthrospore) stage which proved similar to *E. fawcetti* in morphology, temperature requirements, and pathogenicity to walnut, peach, and apricot trees.

Nattrass, in 1933, described and named a fungus, *Hendersonula toruloidea*, which produced both a toruloid and a pycnidial stage on apples, peaches, and apricots in Egypt. Judging from Nattrass' descriptions and illustrations, the California fungus resembles this organism closely. Studies of type specimens of *H. toruloidea* confirmed this view. It is concluded, therefore, that *Exosporina fawcetti* is the conidial stage of *H. toruloidea*.

Under the rules of nomenclature the fungus should be designated *Hendersonula toruloidea* providing Nattrass was first to describe it. Both Fawcett and the writer, however, have mentioned the similarity between the toruloid stage of the California fungus and that of *Torula dimidiata* Penz. described from Italy in 1887. Since Penzig's description is meager, sub-

stitution of the species *dimidiata* for *toruloidea* does not seem justified at this time.

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TOP NECROSIS, A VIRUS DISEASE OF GUAR[1]

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INTRODUCTION

Guar, *Cyamopsis psoraloides* DC., is a native of India, where it is extensively grown as a food and forage crop. Although grown experimentally in the United States since 1903 (8), it recently became of importance as a source of a mucilaginous material found in the seed. Guar normally yields between 500 and 1000 pounds of seed per acre in California and Arizona where most of the commercial crop is grown (7).

In July, 1944, K. Starr Chester observed a stipple necrosis of the young leaves of guar in an experimental planting at Stillwater, Oklahoma. Preliminary infection studies indicated that the disease was caused by a virus. A search of the literature failed to disclose a disease of this nature affecting guar. Following preliminary infection studies, the disease was reported as a new virus disease of guar (2), and was later given the name "top-necrosis" (16).

When first observed on July 7, 1944, less than 0.1 per cent of the plants had definite symptoms. The leaf symptoms were yellowing, stunting, occasionally vein-clearing or a faint oak-leaf pattern, and still more rarely a light chlorotic mottling. By careful observation one could discern a stipple of very small chlorotic depressions on the leaflets. As the leaves became older these chlorotic areas became necrotic. Unequal interference with growth throughout the leaflet often resulted in skewness or rugosity. Many of the more mature leaves became bronzed, with marked intercostal chlorosis.

A count of plants with definite symptoms showed that 2.2 per cent were infected on August 18. Some of the more severely affected plants now had dark brown to black lesions on the upper portion of their stems and on the petioles. These lesions became more pronounced as the season advanced, causing a general necrosis of the stem and growing point of the plant, severe abscission of the younger leaves, and finally, bare stems with only a few chlorotic lower leaves (Fig. 1). By September 26, 40 per cent of the plants were thus affected, and a total of 90 per cent were showing definite symptoms. At this time the disease was causing an estimated loss of at least 75 per cent (2). The rapid increase in the number of af-

fected plants may have been due to development of symptoms in plants previously carrying the virus in a masked condition, or to an increase in transmission by some insect vector.

The object of this study was to identify the virus causing the disease by determining its host range and the symptoms produced on the suscepts, and to investigate its infectivity, transmissibility, thermal inactivation point, dilution end point, and other biological characteristics.

FIG. 1. Four guar plants with various expressions of top-necrosis. Note the blackened necrotic stem of the plant on the left, the defoliation and the curled young leaves of plants in center, and acutely bent stem tip of the plant on the right.

SYMPTOMS OF THE DISEASE ON VARIOUS SUSCEPTS

The following descriptions apply to the disease on several species of plants that were artificially inoculated. Particular attention has been given to those plants commonly used in the diagnosis of virus diseases of legumes. The rubbing method was used almost exclusively in making the inoculations, i.e., the infected plant sap was applied to the upper surface of the leaves with swabs of soft cotton cloth.

On July 12, 1944, 100 plants, 10 each in 10 randomized locations, in the field planting where traces of the disease were first observed, were inoculated with expressed juice from plants in the same field. The first symptoms were observed 9 days later and consisted of a fine chlorotic stipple of the young developing leaflets. By the 12th day a necrotic stipple was the common symptom, with 75 per cent of the plants showing infection. During this time the mean temperature was 80° F. as recorded by a weather station ¼ mile away (6); however, occasional readings showed the actual field temperatures to be much higher. The inoculated plants had 100 per cent infection after 15 days, with most of the plants showing both local and systemic

symptoms, while infection of the noninoculated plants in the field did not exceed 1 per cent. Two months later, 31 per cent of the stem tips were dead.

Guar plants growing out-of-doors in shaded flats were inoculated on August 16. Fifteen per cent of the plants showed well-developed local lesions within 4 days, and systemic symptoms similar to those that developed in the field-grown plants appeared within 6 days. Several different inoculation tests in the greenhouse during the fall and winter gave similar results.

Eight young healthy tobacco plants, *Nicotiana tabacum* L. var. Turkish, growing out-of-doors in 5-in. pots, were inoculated on September 21. Four of these plants developed from 1 to 4 local necrotic lesions each. The initial diameter of the lesions ranged from 3 to 5 mm. The lesions consisted of a thin necrotic ring enclosing slightly chlorotic tissue, which after a time became necrotic throughout.

Under normal greenhouse conditions a total of 25 tobacco plants, which were inoculated at different times, failed to produce symptoms, and subinoculations failed to indicate that the virus was present in masked condition. However, freshly inoculated plants placed under a moistened sheet, where the temperature averaged approximately 68° F., developed watersoaked rings in 8 days. These rings soon became necrotic, surrounding green centers, which slowly became necrotic throughout. No systemic symptoms developed, nor were the young leaves carrying the virus, so far as could be determined by subinoculations. It should be pointed out that several environmental factors were changed when the plants were placed under the moist sheet.

Twelve succulent tobacco plants growing in a floor bed of the greenhouse were topped to a height of 18 in. and inoculated on March 10. Local lesions were observed on 5 of the plants 10 days later, and by the 17th day 11 plants had developed systemic as well as local symptoms. A comparable inoculation made March 11 with tobacco ringspot virus also resulted in severe infections. During this period the greenhouse temperatures ranged between an average daily maximum of 93.5° F. and an average daily minimum of 71.8° F., with a mean of 80.6° F. Probably the vigorous growth of the plants, or the effect of topping them, overcame the inhibitory effect of the high temperatures (14). Later 25 other tobacco plants were inoculated with the guar virus. Local symptoms appeared on 56 per cent, and systemic symptoms on 16 per cent of these plants.

Inoculated Jimson weed, *Datura stramonium* L., developed very small, indefinitely outlined chlorotic areas on the inoculated leaves within 6 days; two days later these had changed to necrotic rings or continuous lesions from 0.5 to 1.0 cm. in diameter. The systemic infection consisted of chlorotic or occasionally necrotic rings or lesions, which by enlargement developed into a chlorotic mottle. The virus could be recovered by sub-inoculations.

The first inoculations of cowpea, *Vigna sinensis* Endl. var. Clay, gave a very small percentage of locally infected plants. In an attempt to find a local lesion host suitable for quantitative studies of the virus, 5 varieties of *V. sinensis* Endl., namely, Clay, Black Eye, New Era, Brown Sugar Crowder, and Chinese Red, and *V. catjang* (Brum.) Walp. var. Catjang were inoculated. A very fine washed sand was used as an abrasive in this test. The varieties Clay, Black Eye, and Catjang developed numerous continuous red lesions 0.5–4.0 mm. in diameter within 5 days after inoculation. The varieties New Era, Brown Sugar Crowder, and Chinese Red formed reddish necrotic rings with more or less normal centers. The diameters of these rings varied with the different varieties, from 0.5–3.0 mm. for Brown Sugar Crowder to 2.0–5.0 mm. for New Era. In all varieties except Clay, necrosis spread along the leaf veins from the point of initial infection. The intercostal tissue of the leaf soon became chlorotic. The leaves had drooped at the pulvinus by the 6th day. Then necrosis, which had spread to the stem by way of the petiole, resulted in death of the plant within 9 to 12 days after inoculation.

Several varieties of bean, *Phaseolus vulgaris* L., were tested and all proved to be only systemically susceptible. In order to make further comparisons, more varieties were tested. The seeds were furnished by the Corneli Seed Company, St. Louis, Missouri, and Asgrow Seed Company, Memphis, Tennessee. The relative number of plants infected and the severity of the disease are shown in table 1. Severity of the systemic symptoms is rated in one of three classes. Class 1 indicated a chlorotic stipple or a very mild necrotic stipple, usually occurring along the margins of the leaflets, sometimes resulting in rugosity. Typical of this group were the Kentucky Wonder selections. Class 2 was characterized by a marked necrotic stippling of the young leaflets which occasionally abscised. An occasional stem tip was killed, especially if the plants were inoculated before the first trifoliate leaves developed. The variety Full Measure typifies this group. Class 3 was similar to the preceding, but with more leaf necrosis and abscission, as in the variety Plentiful. Death of the growing point was common.

The incubation period for systemic infection varied from 6 to 10 days. The local symptoms consisted of necrotic lesions occurring within 4 to 6 days. Necrosis rapidly spread to the vascular system of the leaf and petiole and resulted in a drooping of the leaf at the pulvinus or in a downward curvature of the entire petiole. Even though the percentage of plants developing local symptoms was usually low, the virus frequently killed the plant.

Five varieties developed both local and systemic symptoms. Two varieties, Ideal Market and Genuine Cornfield, developed reddish-brown rings at the points of infection; veinal necrosis spread from this. Similar, although more marked, local lesions appeared on the variety Yard Long, *Vigna sesquipedalis* (L.) W. F. Wright. The symptoms on this variety are characteristic for this genus as described above.

TABLE 1.—*Reaction of bean varieties to guar virus*

Variety and source[a]	Plants with systemic infection		Plants with local infection
	Number infected	Disease rating[b]	Number infected
Tennessee Green Pod (A)	7 of 20	3	
Burpee's Stringless Green Pod (A)	7 of 8	2	
Asgrow's Stringless Green Pod (A)	9 of 10	2	
Landreth Stringless Green Pod (C)	9 of 10	2	
Giant Stringless Green Pod (A)	9 of 10	2	
Giant Stringless (C)	5 of 9	2	
Tendergreen (C)	4 of 10	2	
Tendergreen, New Style (C)	7 of 10	2	
Florida Belle (A)	7 of 21	2	
do (C)	10 of 10	2	
Keystonian (C)	7 of 9	3	
Full Measure (A)	9 of 10	2	
do (C)	8 of 10	2	
Bountiful (A)	5 of 10	2	
do (C)	2 of 10	3	
Plentiful (A)	10 of 10	3	
do (C)	8 of 8	3	
Commodore (C)	2 of 10	2	
Fordhook Favorite (C)	5 of 7	2	
Dwarf Horticultural (C)	4 of 10	3	
Streamliner (A)	5 of 9	2	
do (C)	7 of 9	1	
Asgrow Stringless Black Valentine (A)	8 of 10	2	
Stringless Black Valentine (C)	10 of 10	3	
Black Valentine Round Pod (C)	3 of 10	2	
Red Valentine (A)	3 of 10	3	
do (C)	4 of 10	2	
Red Valentine Stringless (C)	8 of 10	2	
Sure Crop Black Wax (C)	20 of 30	3	
Pencil Pod Black Wax (C)	22 of 22	2	
Davis Stringless Wax (C)	14 of 28	2	
Brittle Wax (C)	17 of 24	3	
Golden Wax Improved (C)	9 of 9	2	
Golden Top Notch (C)	8 of 10	2	
Dark Red Kidney (A)	4 of 10	1	
London Horticultural (A)	3 of 4	3	
McCaslan (A)	8 of 10	3	
Blue Lake Stringless (A)	7 of 17	2	
Kentucky Wonder (A)	13 of 17	1	
do (C)	11 of 19	1	
Kentucky Wonder Wax (A)	11 of 20	1	
Kentucky Wonder Rust Resistant (C)	14 of 20	1	
Alabama Pole No. 1 (C)	10 of 20	1	
Black Stringless Creaseback (C)	10 of 10	2	
Ideal Market (A)	14 of 31	2	10 of 31[c]
Tennessee Wonder (A)	6 of 20	1	20 of 30
Potomac (A)	4 of 12	1	4 of 12
Decatur (A)	4 of 10	2	5 of 10
White Creaseback (C)	20 of 30	2	18 of 30
Genuine Cornfield (A)			21 of 32[c]
Great Northern (A)			12 of 21
U. S. Refugee No. 5 (A)			6 of 18[d]
do (C)			4 of 31[d]
Idaho Refugee (C)			3 of 18
Yard Long (A)			7 of 21

[a] (A) refers to the Asgrow Seed Company; (C) refers to the Corneli Seed Company.
[b] Rating 1, chlorotic or mild necrotic stipple; 2, marked necrotic stipple; 3, leaf necrosis and abscission.
[c] Reddish-brown rings at the infection points.
[d] A few of these plants developed only vienal necrosis of petiole and stem.

Twelve inoculated Lima bean plants, *Phaseolus lunatus* L. var. Baby Potato, developed a few reddish-brown, irregularly outlined, necrotic local lesions, with no systemic symptoms evident. Later three varieties of Lima beans furnished by Corneli Seed Company were also inoculated. Early Bush Market developed numerous local lesions similar to those on the Baby Potato variety. Necrosis spread rapidly along the leaf veins, severely stunting the plants. In Dixie White, small yellow, later necrotic, spots spread along the veins to form irregular brown necrotic areas. The entire leaf soon yellowed, and the plants died within a week or two. Henderson Bush Lima beans developed symptoms similar to, but not nearly so severe as, those on Early Bush Market. The incubation period for all varieties was about 7 days.

Nine of ten inoculated sesame plants, *Sesamum orientale* L., developed within 5 days large necrotic local lesions which rapidly spread throughout the leaf; meanwhile systemic necrosis began to develop in the top of the plant. Five days after local lesions appeared, all of the plants were dead. These results were confirmed by further inoculations.

Petunia, *Petunia hybrida* Vilm. vars. Balcony and Rose of Heaven, developed local and systemic symptoms. The former consisted of small necrotic rings, while the latter were necrotic rings and lines with a severe distortion of the leaves. The virus was recovered from both locally and systemically infected tissue.

Tobacco, *Nicotiana glutinosa* L., did not develop any apparent symptoms, even though numerous inoculations were made; however, sub-inoculations to cowpea var. Black Eye, resulted in 5 lesions on 20 leaves inoculated with sap from symptomless *N. glutinosa* leaves which had developed subsequent to inoculation.

Husk tomato, *Physalis pubescens* L., developed small necrotic rings on the inoculated leaves. No systemic symptoms were observed; however, the virus could be recovered as readily from the young leaves as from the inoculated leaves.

Inoculated green mung bean, *Phaseolus aureus* Roxb., developed no local lesions, but produced a necrosis of the young leaves and stem tips. Only a small percentage of the inoculated plants were infected.

The symptoms on soybean, *Soja max* (L.) Piper, variety S-100, were a systemic stipple necrosis of the young leaves followed by death of the stem tip. Only about 25 per cent of the inoculated plants were infected.

Pea, *Pisum sativum* L. var. Alaska, developed no local lesions but there was a light chlorotic mosaic of the interveinal areas in stunted leaflets developing about two weeks after inoculation.

NON-SUSCEPTS

The following plants did not become infected when inoculated with the guar virus. Sub-inoculations with negative results were made from those species marked with an asterisk. *Leguminosae:* peanut, *Arachis hypogaea*

L.; sesbania, *Sesbania macrocarpa* Muhl.; urd bean, *Phaseolus mungo* L.; adzuka bean, *Phaseolus angularis* (Wild.) W. F. Wright; hairy vetch, *Vicia villosa* Roth.; and crotalaria, *Crotalaria intermedia* Kotschy. *Solanaceae:* pepper, *Capsicum frutescens* L. var. Ruby King*; Jimson weed, *Datura meteloides* L.*; and tomato, *Lycopersicon esculentum* Mill. var. Bonny Best. *Compositae:* zinnia, *Zinnia elegans* Jacq. vars. Giant* and Cut and Come Again; and sunflower, *Helianthus annuus* L. *Amaranthaceae:* globe amaranth, *Gomphrena globosa* L.*

PHYSICAL PROPERTIES

Approximately 18 Giant Stringless Green Pod bean plants were inoculated with each of 12 dilutions of the virus sap between 1:1 and 1:1,000,000. The greatest dilution at which infection occurred was 1:1000. Similar results were obtained in two other tests in which a local lesion host, Black Eye cowpea, was used. In all tests there was a proportionate decrease in infection with dilutions from 1:30 to 1:1000.

Expressed plant sap was put into small test tubes attached to thermometers. Then they were immersed in water which was kept at the given temperature for 10 min. The heating action was checked by plunging the tubes into cool water. In the first test the temperatures used were at 5-degree intervals between 40° and 70° and at 80°, 90°, and 95° C. Approximately 30 Giant Stringless Green Pod bean plants were inoculated with sap from each treatment. Infectivity began to decrease with virus heated to 55° C. A single plant was infected with virus heated to 65° and none with virus heated to 70° C. In the second test the temperatures used were at 2-degree intervals between 57° and 67° C. Approximately 40 primary leaves of Black Eye cowpea were inoculated with juice from each treatment. The average numbers of lesions per leaf were as follows: Check, 19.0 lesions; 57°, 25.9; 59°, 16.3; 61°, 20.8; 63°, 14.6; 65°, 5.2; and 67°, 1.2. Apparently the thermal inactivation point is between 67° and 70° C.

Thoroughly macerated tissue of infected guar plants, with enough water added to make a thin paste, was packed into large test tubes and stored in the refrigerator, some as a chilled liquid and some frozen in the freezing compartment. Under both conditions, the material stored was found to be somewhat infective after 2 months but not after 4 months.

Infected Giant Stringless Green Pod bean leaves were found to lose their infectivity upon drying to a crisp. The extract was made by wetting the leaves with water and macerating them.

The rapid spread of the disease in the guar planting suggests that an insect vector is responsible for its rapid dissemination. In studies of virus transmission through guar seed, 2200 seedlings from seed of infected plants were observed for 35 days, at which time 0.2 per cent of the plants had symptoms similar to those produced by the virus.

COMPARISON WITH SOME KNOWN VIRUS DISEASES

Guar virus symptoms on certain bean varieties are similar to those described by Zaumeyer and Harter (18) for bean mosaic viruses 4 and 4A. However, other varieties differentiate between these and the guar virus. Likewise the guar virus infects cowpea while the bean viruses do not. The thermal inactivation point for the bean viruses is 95° C. compared to 68° C. for the guar virus.

Holmes (3) recognizes 7 strains of cucumber mosaic virus. Six of these produce dark red necrotic lesions on Black Eye cowpea (9) that are similar to those caused by the guar virus. However, 5 of these, *Marmor cucumeris* vars. *vulgare, commelinae, phaseoli, lilii,* and *judicus* Holmes, infect zinnia and may be recovered from it by sub-inoculation (10), while the guar virus failed to infect zinnia. The 6th strain, *M. cucumeris* var. *upsilon,* is inactivated by exposure at 52° C. for 10 min.

The "S" strain of potato virus "X" (*Marmor dubium* var. *annulus* Holmes) produces ringspot symptoms on tobacco (12). However, pepper is highly susceptible to this potato virus but was not infected with the guar virus in repeated inoculations.

A virus disease has been observed on commercial bean varieties in Louisiana and Mississippi with symptoms on several hosts and certain physical properties that are very similar to those of the guar virus (5).

The similarities between the symptoms of the guar virus on bean and tobacco and those reported for tobacco ringspot were such that direct comparisons were considered necessary. To make this possible, Dr. W. C. Price kindly furnished inoculum of the tobacco ringspot virus described by Wingard (17). In comparative inoculations of the two viruses on guar, there was no development of systemic lesions on the young leaves of the plant inoculated with the tobacco ringspot, although stem necrosis began at the point of petiole attachment of the inoculated leaf.

Comparative inoculations of sesame with the two viruses resulted in very similar symptoms, both being rapidly lethal. Likewise, Black Eye cowpea receiving comparative inoculations developed very similar symptoms from the two viruses, the local lesions caused by the guar virus being slightly larger.

Comparative inoculations were made on vigorous Turkish tobacco plants growing in a floor bed of the greenhouse. The local lesions on the plants inoculated with the guar virus consisted of very narrow necrotic rings, with many very fine concentric secondary lines forming about the initial ring. This gave the entire lesion a diffuse appearance. The lines were much wider and with fewer secondary rings on the plants inoculated with the ringspot virus. There were few qualitative differences between the systemic symptoms of the two viruses (Fig. 2). The systemic necrotic rings tended to develop on the guar virus infected plants for a longer period, and the color intensity of the recovered leaves was less than in

leaves similarly infected with the tobacco ringspot virus. The mean bi-hourly temperature in the greenhouse was 80.6° F. for a 20-day period following inoculation.

Valleau (14) has stated that plants systemically infected with tobacco ringspot produce a reduced amount of pollen and that a large percentage of the pollen grains are smaller than normal. Later (15) he found this to be true for 6 different strains of the virus. Representative samples of pollen from plants systemically infected with each of the two viruses were mounted in lacto-phenol with dilute acid-fuchsin as a stain. Measurements were made with an oil immersion objective and ocular mi-
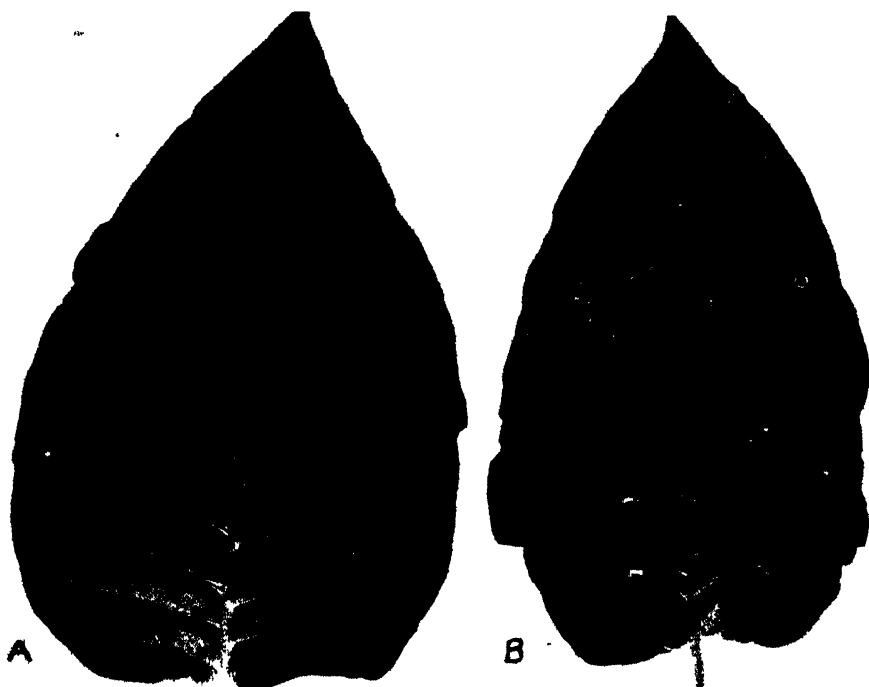
FIG. 2. Comparison of systemic symptoms of guar virus (A) and tobacco ringspot virus (B) on Turkish tobacco. Note the differences in shading of the areas inside and outside of the oak-leaf outline, and in the thickness of the necrotic lines. (A × ½, B × ⅓)

crometer with unit spaces equivalent to 1.37 microns. The number of plasmolyzed grains in each field was recorded. The results (Table 2) show a close similarity between the two groups.

Considering the different viruses producing ringspot symptoms on tobacco, the following characteristics will differentiate between them and the guar virus. The Bergerac ringspot virus, *Annulus bergerac* H., is, in addition to differences of symptoms, inactivated at 80° C. in 10 min. (13, pp. 285–289). Tobacco streak-disease virus, *A. orae* H., also differs in several respects, the greatest being that it is inactivated at 53° C. (4). Tomato ringspot, *A. zonatus* H., is inactivated by temperatures of 55–60° C. The guar virus did not infect tomato.

Three strains of tobacco ringspot are recognized by Holmes (3). The typical strain, A. tabaci var. virginiensis H., produces local lesions on bean, is more severe on tobacco, and is not typically systemic on guar. The yellow strain, A. tabaci var. auratus H., causes a definite chlorosis of systemically infected tobacco plants (14). The green strain, A. tabaci var. kentuckiensis H., is not highly lethal to cowpea when compared to the typical strain (11), whereas the guar virus was just as lethal as the typical strain of tobacco ringspot.

The author had thought that perhaps the guar virus, the soybean bud blight virus, and the bean virus studied by LeBeau were all a variant of tobacco ringspot virus that was highly infective to legumes. However, Allington (1) recently demonstrated the soybean bud blight virus to be

TABLE 2.—*Comparative measurement of Nicotiana tabacum pollen grains from plants systemically infected with tobacco ringspot and guar viruses*[1]

Diameter (microns)	Ringspot Virus		Guar Virus	
	Frequency	Percentage	Frequency	Percentage
Plasmolyzed[2]	71		64	
20.55	1	0.38	4	0.51
21.92	5	1.88	7	2.64
23.29	7	2.63	18	6.79
24.66	22	8.27	35	13.21
26.03	34	12.73	42	15.85
27.40	77	28.95	53	20.00
28.77	83	31.20	53	20.00
30.14	33	12.48	35	13.21
31.51	4	1.50	11	4.15
32.88			3	1.13
34.25			2	0.75
35.62			1	0.38
36.99			1	0.38
Total	266	100.02	265	100.00

[1] Measurements made May 13, 1945, from greenhouse grown plants.
[2] The plasmolyzed grains were not included in calculation of percentages and totals.

identical with the typical strain of tobacco ringspot virus when compared on tobacco, Red Kidney bean, and cucumber (*Cucumis sativus* L.).

It is concluded that host ranges, symptoms, and physical properties of the guar virus and the tobacco ringspot virus show a relationship. However, since certain differences do exist, the guar virus is considered to be a new and distinct variety of tobacco ringspot virus, for which the the name *Annulus. tabaci* Holmes var. *cyamopsidis* is proposed.

SUMMARY

An undescribed virus was found naturally infecting guar, *Cyamopsis psoraloides* DC. The infections increased in the field from less than 0.1 per cent to more than 90 per cent in 2½ months.

The virus was sap-transmissible to bean, Lima bean, soybean, cowpea,

green mung bean, tobacco, *Nicotiana glutinosa* L., husk tomato, Jimson weed, garden pea, and sesame. It failed to infect peanut, sesbania, urd bean, adzuka bean, hairy vetch, crotalaria, pepper, *Datura meteloides* L., tomato, zinnia, sunflower, and globe amaranth.

It was systemically infective to 42 varieties of bean, 5 of which also showed local lesions and veinal necrosis. Six varieties developed only local lesions and veinal necrosis. On 6 varieties of cowpeas the virus produced local lesions from which necrosis progressively spread, becoming lethal in 5 of them.

The thermal inactivation point was approximately 68° C. for 10 min. exposure. The dilution end point was 1:1000 and the virus was inactivated in air dried tissue. Macerated infected tissue retained some of its infectivity for 2 months when kept refrigerated at 5° C. or frozen. It was non-infective after 4 months storage.

Comparative studies indicated that pollen grains developed in completely infected tobacco plants were reduced in size and uniformity by the guar virus as well as the tobacco ringspot virus. A very small percentage of seed from infected guar plants apparently transmitted the guar virus.

There are many points of similarity between the typical tobacco ringspot virus and the guar virus. However, the following differences do exist: 37 of 48 varieties of bean failed to produce local lesions as is typical of tobacco ringspot. The guar virus did not infect zinnia and its symptoms are milder on tobacco than those of the typical strain of tobacco ringspot virus. The latter also produced different symptoms on guar

A comparison of host ranges, the symptoms on various suscepts, and the physical characteristics of tobacco ringspot virus and the guar virus shows a close relationship. However, the differences are believed to be such as to warrant considering the guar virus a new and distinct variety of the tobacco ringspot virus, for which the name *Annulus tabaci* Holmes var. *cyamopsidis* is proposed.

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CULTURAL VARIATION, TAXONOMY AND PATHOGENICITY OF FUSARIUM SPECIES ASSOCIATED WITH CEREAL ROOT ROTS[1]

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INTRODUCTION

To determine the causal fungi associated with cereal root rots[3] in California, and their relative frequency, numerous isolations were made over a four-year period. A large proportion proved to be Fusaria. Because of their evident importance in the root rot complex, an investigation of the variation, pathogenicity, and taxonomic relationships of the Fusarium isolates was undertaken.

Species of *Fusarium* in Wollenweber and Reinking's (21) sections Discolor, Roseum, and Gibbosum were encountered primarily and attention was focused upon these. In these sections according to Dickson (4), pp. 207–209, only three species are generally considered to be of primary importance as cereal root rot pathogens, *Fusarium graminearum* Schw. and *Fusarium culmorum* (W. G. Sm.) Sacc. of the section Discolor and *Fusarium avenaceum* (Fr.) Sacc. of the section Roseum. In the section Gibbosum, Bennett (2) and Johnston and Greaney (12) have demonstrated *Fusarium equiseti* (Cda.) Sacc. to be weakly pathogenic, as have Gordon and Sprague (7), Sprague (18), and Bennett (1) for certain varieties and strains of *Fusarium scirpi* Lamb. et Fautr.

Cultural variation is a common phenomenon in the cereal Fusaria. Eide (5), Goddard (6), Ullstrup (20), and Tu (19) have reported variants in *F. graminearum*. Tu (19) likewise demonstrated variants in *F. culmorum* and *F. avenaceum*. Variation in *F. culmorum* and *F. sambucinum* was shown by Mitter (13).

That variation in pathogenicity accompanies cultural variation has been shown by Ullstrup (20), Eide (5), and Tu (19). All have demonstrated cultural variants to be less pathogenic than the parental type. Johnston and Greaney (12) found, in nature, isolates of *F. culmorum* ranging from severe parasites to practical saprophytes, some even less pathogenic than *F. equiseti* and *F. avenaceum*.

Positive identification of species and forms within the genus *Fusarium* has been difficult and usually is undertaken only by those having special

[3] In this paper root rot is used in its broadest sense to include injury to root, crown basal culm, and leaf sheath tissue.

knowledge of their characteristics and relationships. The problem of taxonomy is complicated by the numerous cultural variants that appear and by the diversity of forms that occur in nature.

The standard taxonomic system has been that contained in Wollenweber and Reinking's monograph, *Die Fusarien* (21), in which the genus is divided into 16 sections each containing species and forms. Evidence that taxonomic lines drawn by this work have been too fine for practical usage has been brought forth by Hansford (11), Snyder and Hansen (14), Mitter (13), and Brown (3). Some investigators have observed that variants of a given species cross not only species lines but in some cases sectional groupings (13, 17). Snyder and Hansen (14, 16, 17), in pointing out the unreliability of such key characters as conidial size and shape, presence and type of chlamydospores, and stromatal color, have completed an extensive revision of the genus reducing the species to nine. They propose the use of host relationships as bases upon which to separate the pathogenic forms within morphological units. In the cereal Fusaria they have reduced all species, varieties and forms of the sections Discolor, Roseum, and Gibbosum[1], to the species *Fusarium roseum* (Lk.) Sny. and Hans. All forms pathogenic on cereals are denoted as *F. roseum* forma *cerealis*.

The investigations reported here were undertaken (1) to find the range of variability in the *Fusarium* species which had been isolated in pure culture, using the single spore technique throughout; (2) to obtain evidence on the question of reversion of variants to the parental type; (3) to compare the cultural variants obtained in these studies with their parent types, and to determine the species identity of each variant independently of any knowledge of the identity of the parent type from which it came; (4) to compare the pathogenicity of the different species of *Fusarium* isolated and the pathogenicity of the parent types with that of the cultural variants; and (5) to obtain a clearer evaluation of the relationships of the Fusaria that cause root rot of cereals.

CULTURAL STUDIES

Methods.—To initiate each isolate in pure culture, twenty of the first conidia produced on the mycelium advancing from the diseased tissue were isolated singly. When perithecia were available, single ascospore cultures were made directly.

Except where specified, isolates were transferred by employing single conidia as inocula following the method used by Hansen and Smith (9). Large test tube slants of potato-dextrose agar were used as media throughout. All cultures were maintained at room temperatures (23°–25° C.) and exposed continuously to diffuse daylight. Sporulation was more profuse and normal under these conditions, an observation in agreement with that of

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[4] Wollenweber and Reinking (21) separate these 3 sections as follows: species in the section Roseum lack chlamydospores; those in the section Gibbosum have intercalary chlamydospores; and those in the section Discolor have intercalary and sometimes terminal chlamydospores.

Snyder and Hansen (15). To avoid the possibility of culture mite contamination, the tubes were capped with cigarette paper as described by

TABLE 1.—*Sources of isolates of Fusarium species, their cultural type, and their relative pathogenicity on wheat seedlings*

Species designation of field isolate	Isolate	Origin	Cultural type[a]	Disease rating[b]
Section Discolor				
F. graminearum	3	wheat	I	2.5
Do	4	wheat	I	4.8
Do	4A	culture variant of 4	II	2.0
Do	5	barley	I	3.1
Do	6	wheat (perithecia)	I	4.3
Do	6A[c]	culture variant of 6	II	0.7
Do	7	wheat	II	0.9
Do	11	corn	I	5.0
Do	13	barley	I	3.9
F. culmorum	1	barley	I	3.7
Do	2	barley	I	3.1
Do	3	barley	I	3.6
Do	3A	culture variant of 3	I	2.1
Do	4	wheat	I	4.0
Do	4A[d]	culture variant of 4	II	0.1
Do	5	corn	I	4.5
Do	5A[e]	culture variant of 5	II	0.1
Do	7	barley	I	2.8
Do	7A[f]	culture variant of 7	II	1.3
Do	7B[g]	culture variant of 7	II	0.8
F. sambucinum	1	barley	I	0.0
Do	2	Stipa sp.	I	0.0
Do	2A	culture variant of 2	II	0.0
Section Gibbosum				
F. equiseti	1	wheat	I	0.5
Do	2	wheat	I	0.7
Do	2A[h]	culture variant of 2	II	0.3
F. scirpi	1	wheat	I	0.0
Do	2	Stipa sp.	I	0.0
Section Roseum				
F. avenaceum	1	peas	I	1.0
Do	1A	culture variant of 1	II	0.4
	Check			0.0

[a] Type I is a culture with abundant aerial mycelium with conidia borne in sporodochia; Type II cultures are appressed with numerous conidia in pionnotes.

[b] Three replications of 15 wheat plants each, variety Baart 38, were used to test each isolate. The plants were grouped into 6 classes from 0 to 5 increasing with severity of the disease (0 = healthy; 5 = dead), and a disease rating was calculated for each replicate. Significant differences between average disease ratings; 5 per cent level, 0.7: 1 per cent level, 1.0.

[c] Variant 6A reclassified as *Fusarium sublunatum* var. *elongatum* Rg.

[d] Variant 4A reclassified as *Fusarium flocciferum* Corda.

[e] Variant 5A reclassified as *Fusarium reticulatum* Mont. f.1 Wr.

[f] Variant 7A reclassified as *F. culmorum* var. *cereale* (Cke.) Wr.

[g] Variant 7B reclassified as *F. sambucinum.*

[h] Variant 2A could not be fitted into existing classification.

Hansen and Snyder (10). Spore measurements were made under high power of the microscope with a filar micrometer.

Sources of isolates.—In table 1 the isolates and variants selected for these investigations are listed together with their origin, cultural type, and disease rating. As each isolate was obtained from nature it was designated with the binomial in Wollenweber and Reinking's *Die Fusarien* (21) that appeared to fit it most accurately. No isolate having the characteristics of the section Roseum was obtained from California cereals. Some isolates originally placed in this section later had to be considered as belonging to Gibbosum. A culture from pea roots from Hamilton, Montana, was used to represent this group. The isolates studied include three species of the section Discolor, two of the section Gibbosum, and one of the section Roseum.

Cultural type of field isolates.—From the initial single-spore isolations from naturally diseased tissue two types of cultures resulted, an expression of the Hansen (8) dual phenomenon. Throughout the rest of the studies these two were evident in all six Fusarium species investigated. They will be referred to as Types I and II hereafter.

Type I is characterized by an abundance of aerial mycelium often filling the culture tube. The conidia are ordinarily produced in sporodochia but occasionally are found sparsely scattered in groups in the aerial mycelium.

Type II has little or no aerial mycelium, the organism growing closely appressed to and in the agar. As cultures of this type age, they usually take on a slimy appearance. Conidia are produced in great abundance in pionnotes or sometimes in pseudopionnotes.

The letters C and M as used by Hansen (8) to designate the two cultural types of fungi are not used as they are not applicable to the cereal Fusaria. In Hansen's work with Fusaria of the sections Elegans and Martiella, the primary type was conidial (C) and the secondary, mycelial (M). In the cereal Fusaria the primary culture type (I) has abundant aerial mycelium and the secondary form (II) is pionnotal rather than mycelial. Type I appears to correspond to Eide's (5) Types A-H and Ullstrup's (20) Type A, and Type II to Eide's Type I and Ullstrup's Type B as described in their respective studies with *F. graminearum.*

Of 390 conidia of 5 species of *Fusarium* isolated singly from nature, 365 were of culture Type I. In the instance of isolate 7 of *F. graminearum* all 20 initial single conidia produced colonies of the pionnotal Type II. This indicates the organism probably occurred in nature in that form. An isolate of *F. sambucinum* yielded cultures of both types in equal numbers. Perithecia of *Gibberella zeae* (Schw.) Petch were available in one case and 40 single ascospores produced colonies exclusively of Type I. The results are in accord with those of Eide (5) and demonstrate that although the sporodochial Type I is predominant, the pionnotal Type II does appear to occur in nature. Furthermore, a similar situation was met with in *F. culmorum, F. scirpi, F. equiseti,* and *F. sambucinum.*

Variation in culture.—Twenty-seven different isolates, comprising six species of *Fusarium,* were analyzed through 3 to 4 generations by employing the following single-spore method: The original single-spore isolates were

kept at least 8 weeks, and in most cases over a year, before the second single-spore generation was initiated in order to allow time for maximum variation in the original single-spore cultures. At least 10 single spores were isolated from the original single-spore culture. If all of the cultures in the second generation were identical as to cultural characters only one was carried into the third generation. If, on the other hand, variants occurred along with the parental type, both were continued into the next generation. Each culture was carried through at least three generations by this technique.

Of the approximately 600 single-spore transfers of isolates representing the six species of *Fusarium*, the resulting cultures always could be placed with ease into either cultural Type I or Type II. There was variation within the Type I and Type II cultures as to color, amount of mycelium and
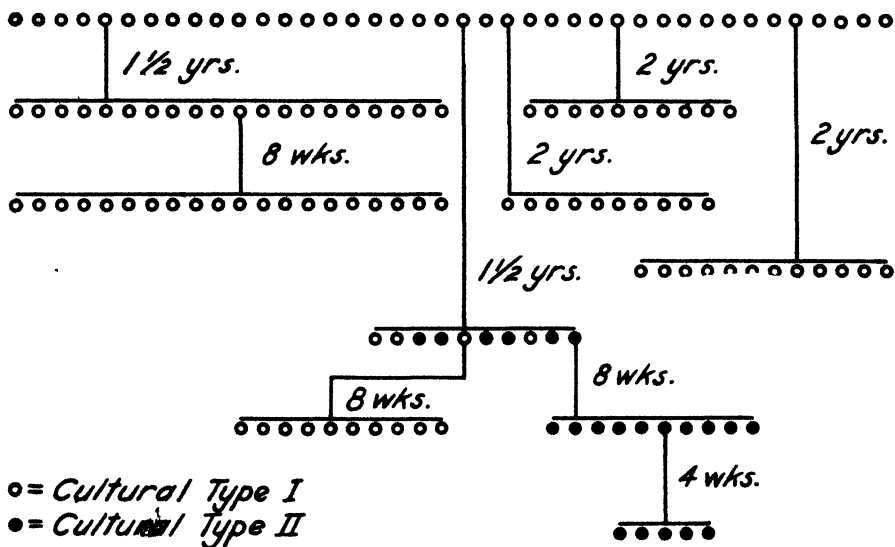
FIG. 1. Single-spore analysis of an isolate of *F. graminearum* (*Gibberella zeae*). Each circle in the first generation represents a single-ascospore culture direct from nature. Thereafter each circle represents a single-conidium culture. Time intervals between transfers are indicated along lines connecting generations. Type I refers to the sporodochial type most commonly isolated from nature and II to the pionnotal variant. (See fig. 3.) Of the 5 ascospore cultures carried into the second generation, one mutated to the pionnotal type and remained constant thereafter.

sporulation, but there was always a definite distinction between the two general types.

In each of the six species there were isolates that gave rise to Type II pionnotal variants during the course of single-spore transfer. Three of 13 isolates of *F. graminearum*, and 3 of 7 of *F. culmorum* yielded cultural variants of Type II. In each *F. sambucinum*, *F. scirpi*, *F. equiseti*, and *F. avenaceum* variants of this type were encountered.

A typical analysis of a culture of *F. graminearum* is illustrated in figure 1. Of the five single-ascospore cultures of *Gibberella zeae* carried into the second generation one yielded colonies of the Type II variant as well as the

parent type. This variant remained true to type through two subsequent single-spore generations.

Figure 2 diagrams the cultural behavior of an unstable isolate of *F. equiseti*. After 16 weeks in culture, one of the original single-spore isolates gave rise to 18 Type II variants among the 20 single-spore transfers. These remained constant thereafter. The parental type always yielded a large percentage of variants if sufficient time elapsed between generations. Figure 2 indicates that a period of over 3 weeks was necessary for this variation to occur under ordinary laboratory light and temperature conditions.

There appears to be a general directional change in the cereal Fusaria from the primary Type I, most commonly recovered in nature, to the sec-

o = *Cultural Type I*   ● = *Cultural Type II*
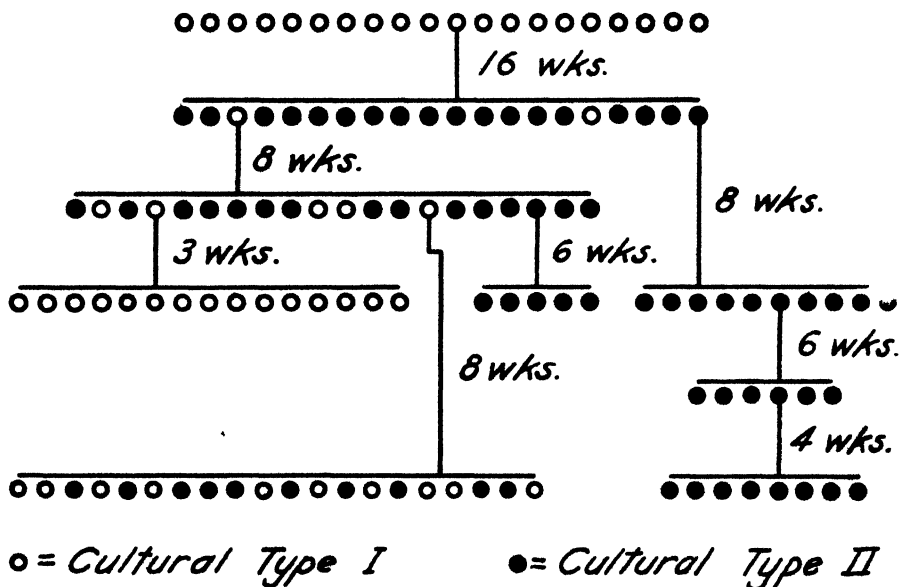
FIG. 2. Single-spore analysis of an isolate of *Fusarium equiseti*. Each circle represents a single-conidium culture, the top row being the original field isolates. Time intervals between transfers are indicated. Type I refers to the sporodochial type as isolated direct from nature and II to the pionnotal variant. In this case Type I is very unstable, continually producing variants of the pionnotal type, all of which remained constant. The effect of time elapsed between transfers is shown in the Type I culture transferred at the age of 3 weeks when no pionnotal types appeared, and at 8 weeks when one half of its spores were the pionnotal mutant.

ondary Type II, as the organism is propagated in culture. This tendency has been observed by others. Ullstrup (20), in studying two isolates of *F. graminearum* by the single-spore method, showed this change, yet the original types were retained as was the case here. Also isolates of the same species differ in the frequency with which they yield Type II variants.

Figure 3 illustrates cultures of *F. graminearum* and *F. culmorum* as originally isolated, together with their corresponding Type II variants.

Constancy of the pionnotal variants in culture.—Controversy exists in the literature concerning the question of reversion of cultural variants to
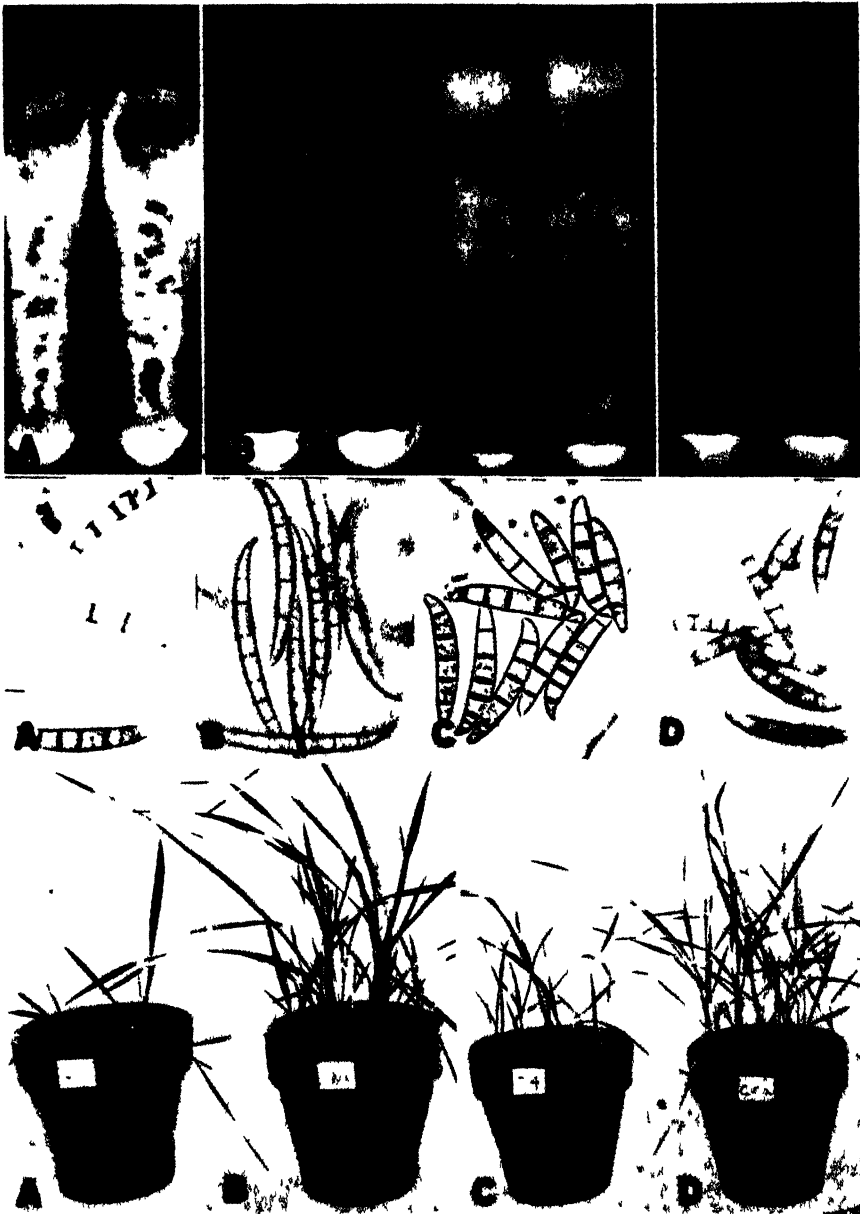
FIG. 3. Comparison of parent and variant single spore isolates of *F. graminearum* and *F. culmorum* on cultural, morphological and pathogenic bases. A. Parent culture 6 of *F. graminearum*. B. Variant 6A of A reclassified as *F. sublunatum* var. *elongatum*. C. Parent culture 4 of *F. culmorum*. D. Variant 4A of C reclassified as *F. flocciferum*. The top row shows test-tube cultures of the two parents and their variants. Each parent is of the sporodochial Type I, whereas the variants are of the pionnotal Type II. The second row illustrates macroconidia (×450) of each culture. Upon these differences in size of the conidia and other features the variants were no longer considered as the same species as their respective parents. The bottom row shows the marked virulence on wheat seedlings of the two parent types as compared to their pionnotal variants.

the parental types. Working with the same fungus, *F. graminearum*, Goddard (6) reported reversion while Eide (5) and Ullstrup (20) found no evidence of it. Ullstrup (20) demonstrated by the use of the single-spore method of transfer that the pionnotal variant remained constant through ten generations.

In this work the writer tested 12 pionnotal variants of five different species. Five Type II variants of *F. graminearum*, 4 of *F. culmorum*, and one each of *F. equiseti*, *F. sambucinum*, and *F. avenaceum* were carried through 1 to 4 generations by single-spore transfer over a period of
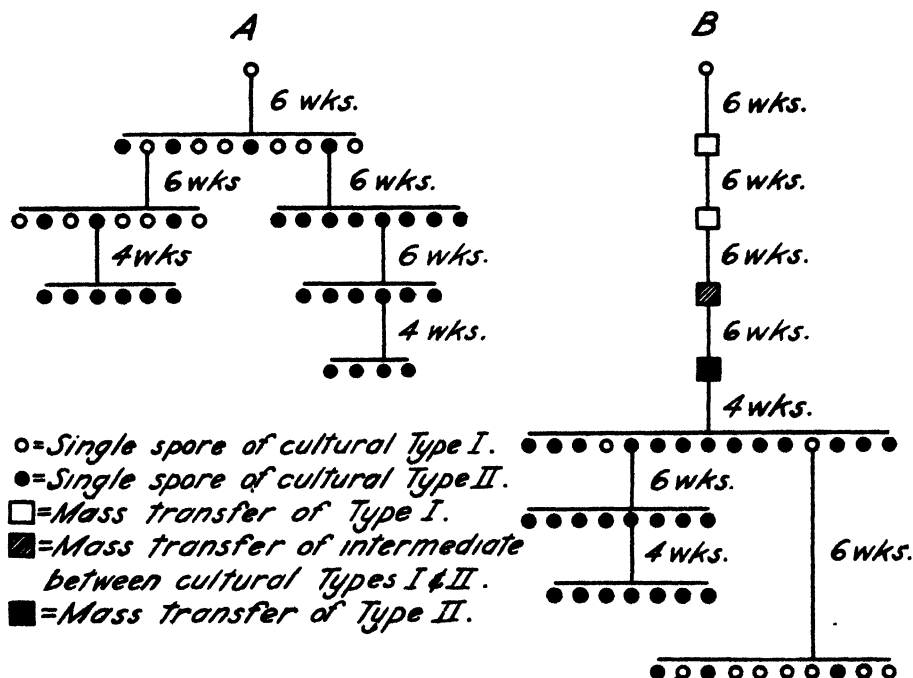
FIG. 4. Diagram of two methods of artificially perpetuating a culture of *F. equiseti*. The two top circles represent single-conidium cultures of the original field isolate. In procedure A, single-spore transfers were made throughout. In B, the original single-spore culture was mass transferred four times and the fourth mass transfer was then single-spored. Type I refers to the sporodochial type from nature and II to the pionnotal variant. Note that by the single spore method, procedure A, no reversion of Type II to Type I occurs, whereas by mass transfer procedure B, an apparent reversion appears.

years. Of 278 single conidia isolated from Type II cultures, all resulting colonies were exclusively of Type II.

The apparent disagreement seems to be rooted in the methods employed in the various investigations. Goddard (6), using mass transfer methods, obtained reversion, whereas Ullstrup (20) and the writer, employing single-spore subculturing exclusively, obtained no evidence for it. An experiment was devised to compare the method of mass transfer with that of the single-spore technique using the same isolate. The procedures are diagrammed in figure 4.

A Type I culture of *F. equiseti* was selected as it very readily produced a Type II pionnotal variant in culture. Two identical single-spore cultures of Type I from the original field isolate were selected. One was subjected to procedure A, the single-spore method of transferring. In the first generation, four of the ten cultures were of Type II. Three subsequent single-spore generations of this Type II showed it to be constant with no evidence of reversion to Type I. In procedure B, the original single-spore culture was mass transferred four generations in succession. That is, a mass of mycelium was taken at the end of six weeks from the original single-spore culture and transferred to fresh medium. At six-week intervals this was repeated as illustrated in figure 4. The culture resulting from the third mass transfer was materially different from the original type. It had scattered tufts of aerial mycelium, but in general it was of Type II. At this stage the culture obviously consisted of a mixture of Types I and II. The culture after the fourth transfer was wholly Type II in appearance and was identical to the Type II cultures produced in procedure A from single spores. Yet upon isolating 15 single conidia from it, two colonies identical to the original Type I appeared. This lends evidence that real reversion does not occur when the variant cultures are always initiated with single spores; yet when mass transfers are used a false reversion can occur which is probably due to a reassortment of nuclei perpetuated from the outset.

Comparison of the variants with the parent cultures from a taxonomic standpoint.—It has been the tendency of workers to consider a cultural variant as a member of the parent species without attempting to fit it into the taxonomic scheme independently of the parent. Mitter (13) pointed out that certain variants of *Fusarium culmorum* had conidia that differed so much from those of the parent in regard to shape that they could not be considered as belonging in the same species or even in the same section as the parent.

The object of this study was to compare the pionnotal variants (Type II) with parental types (Type I) to see whether or not the parent and its variant, when classified independently of one another according to the criteria set up by Wollenweber and Reinking (21), as was done in section Elegans by Snyder and Hansen (14), would fall into the same species. This was done with representatives of four species. The characters used by Wollenweber and Reinking (21), such as the presence of chlamydospores, spore size and septation, and stromatal color, were studied. It was found that chlamydospores in some species occurred more commonly in water culture than on potato-dextrose agar, so both of these methods were employed.

Table 2 summarizes the results of this comparison. In the last column the variants are given the species names, the description of which most closely fits them in *Die Fusarien* (21).

TABLE 2.—*Comparison of Fusarium variants with their parent cultures from a taxonomic and pathogenic standpoint*

Species and Isolate	Cultural Type[a]	Average spore size (5 septate)	Average spore septation	Chlamydospores[b]		Disease rating[c]	Identification of parent and variant cultures[d]
				On potato dextrose agar	In water culture		
Section Discolor							
F. graminearum	6	$51.7 \times 5.3\ \mu$	5	0	0	4.3	F. graminearum
Variant	6A	$62.4 \times 4.9\ \mu$	7	++	+++	0.7	F. sublunatum var. elongatum
F. culmorum	4	$40.4 \times 6.6\ \mu$	5	+	+++	4.0	F. culmorum
Variant	4A	$35.9 \times 4.5\ \mu$	5	-+	+++	0.1	F. flocciferum
F. culmorum	7	$41.0 \times 6.7\ \mu$	5	+	++	2.8	F. culmorum
Variant	7A	$41.5 \times 5.7\ \mu$	5	+	+++	1.3	F. culmorum var. cereale
Variant	7B	$38.4 \times 4.9\ \mu$	5	++	+	0.8	F. sambucinum
F. culmorum	5	$36.2 \times 6.6\ \mu$	5	++	+++	4.5	F. culmorum
Variant	5A	(3 septate) $28.4 \times 5.5\ \mu$	3	+++	+++	0.1	F. reticulatum f.1
Section Gibbosum							
F. equiseti	2	$41.9 \times 5.9\ \mu$	3	0	++	0.7	F. equiseti
Variant	2A	$56.2 \times 3.8\ \mu$	5	0	++	0.3	new species[e]
F. scirpi	1	$45.0 \times 5.0\ \mu$	5	++	+++	0.0	F. scirpi
Variant	1A	No conidia		0	0	0.0	new species[f]
Check				—	—	0.0	

[a] I represents sporodochial type usually isolated from nature; II represents pionnotal variant.
[b] Number of plus signs indicate relative abundance of chlamydospores.
[c] Ranging from 0 to 5 with increasing severity of disease as measured on wheat seedlings. Difference required for significance at 5 per cent level, 0.7—at 1 per cent level, 1.0.
[d] Identification was made by use of the system of Wollenweber and Reinking (21).
[e] This variant could not be placed in the existing system and a new species or variety would have to be created.
[f] This fungus, due to its absence of chlamydospores, can no longer be placed in F. scirpi nor even in the section Gibbosum; it must be considered as being in the section Roseum.

F. graminearum isolate 6, representing the parent Type I, fitted the species description both as to size of conidia and absence of chlamydospores. The Type II variant 6A, however, had much longer and thinner spores with an average septation of 7. In figure 3 the difference between the macroconidia of the parent and variant is shown. Furthermore, the variant contained chlamydospores, which were extremely abundant especially when the culture was grown in water. The length of the macroconidia and the abundance of chlamydospores forbids its classification as *F. graminearum*. Rather it seems to fit *F. sublunatum* var. *elongatum*.

FIG. 5. Comparison of macroconidia of parent and variant isolates of two Fusarium species. A, Macroconidia of *F. culmorum* parent culture 7; B, macroconidia of one of its variants, 7A, reclassified as *F. culmorum* var. *cereale;* and C, macroconidia of its other variant, 7B, reclassified as *F. sambucinum*. D, Macroconidia of *F. equiseti* parent culture 2; and E, macroconidia of its variant, 2A, for which there is apparently no existing species in the section Gibbosum (×450). This difference in the macroconidia of the parent and variant type is one of the factors that necessitates the reclassification of the variants, if the system of Wollenweber and Reinking is to be adhered to closely.

In the three *F. culmorum* isolates studied, four Type II variants appeared, two from one parent culture. While the three parent cultures all fitted the species description, the variants fell into four different places in the taxonomic scheme. One fitted into *F. flocciferum*, another into *F. reticulatum*, and of the two from the same parent, one was *F. culmorum* var. *cereale* and the other, *F. sambucinum*. The variants differed from *F.*

culmorum primarily in their spore width. Figures 3 and 5 illustrate these differences in the macroconidia.

Isolate 2 of *F. equiseti* was originally considered as a member of section Roseum because on potato-dextrose agar no chlamydospores were observed. However, when the fungus was allowed to grow in a water blank for a week, the chlamydospores were produced in abundance. Therefore, it was placed in the section Gibbosum and identified as *F. equiseti*. The Type II variant, 2A, likewise produced chlamydospores only in water, but the conidia were markedly different from those of the parent in length and septation, as shown in table 2 and figure 5. There was apparently no previously described species or variety that would fit this particular variant and a new one would have to be created if this were to be classified in the Wollenweber system.

In the study of the *F. scirpi* isolate, a variant appeared which remained in Type I but had less aerial mycelium and no conidia. This variant completely lacked chlamydospores and therefore had to be removed from the section Gibbosum and placed in the section Roseum. The parent, on the other hand, with its characteristically curved conidia and knots of resting spores, was typically a member of the section Gibbosum.

A taxonomic difference between the variant and parent does not always occur. Culture 4 of *F. graminearum* produced a pionnotal Type II variant culture, 4A, which on the basis of spore measurement and lack of chlamydospores differed in no way from its parent.

PATHOGENICITY STUDIES

Methods.—To study pathogenicity of the Fusarium isolates and their variants, greenhouse tests were employed. Several preliminary trials were conducted with many of the isolates to determine the best host. Wheat proved to be the most satisfactory, being generally more susceptible than barley. Oats were rarely affected. In these early trials different methods of artificial inoculation were used. The application of heavy spore suspensions to the seed or into the open furrows at planting was successful with the abundantly sporulating isolates. This method, however, could not be used in any trial that included all the isolates, for many sporulated only sparsely or not at all.

Soil inoculation with infested oat kernels was used because a standard amount of inoculum could be employed for all the fungi tested. It was recognized that the amount of inoculum used or inoculum potential could strongly affect the apparent pathogenicities of most of the isolates that showed any degree of parasitism. An attempt was made to discover the optimum inoculum potential, *i.e.*, a standard amount of inoculum that would adequately segregate the severe, moderate, and weak parasites.

Having determined the best host plant and the most satisfactory inoculum potential, a replicated experiment including all the isolates and variants was set up. From a single-spore culture of each isolate, transfers

were made in triplicate to three tubes of moist sterilized oat kernels. Each tube was made up with 15 grams of dry oats and 20 cc. of distilled water.

When infestation of the oats was complete, the contaminated kernels from each tube were mixed in the sterile soil of a 6-in. pot. Sterile oats were similarly added to each check pot. After allowing 3 days for the fungi to become established, fifteen seeds of wheat, variety Baart 38, were planted in each of the 3 pots for each isolate tested.

During the trial the soil temperatures ranged from 22° to 28° C., averaging 25° C. At regular intervals approximately equal quantities of water were added to all pots to maintain a soil moisture level favorable for plant growth.

At the end of 24 days, the plants were dug, washed, and grouped into 6 classes ranging from 0 to 5, according to the severity of the disease. Both top and underground symptoms were taken into account. The 0 represented a healthy vigorous plant with no discoloration of the crown or roots. Class 1 plants were those that were apparently healthy in regard to top symptoms but which had definite brown lesions on the crown. These lesions were only in the leaf sheath, not penetrating through to the culm. Class 2 plants were somewhat stunted, having rather severe rotting and discoloration of the basal leaf sheath, culm, and sub-crown internode. Class 3 represented plants definitely stunted and containing a severe rotting of the crown. Plants that were placed in class 4 were those that barely emerged and were dead, or nearly so, by the time of harvesting. Class 5 represented plants that were killed prior to emergence.

A mean disease rating was calculated for each replicate by multiplying the number of plants in each class by the class index number, summing the products, and dividing by the total number of plants. An analysis of variance was then applied to the average disease ratings of the 30 isolates and the check which showed that a difference in disease rating of 0.7 was required for significance at the 5 per cent level and 1.0 at the 1 per cent level.

Experimental Results.—In preliminary trials with small groups of isolates, great variation in pathogenicity was observed among the isolates of the six Fusarium species. In particular, the variants of cultural Type II showed a much lower order of parasitism than their respective Type I parent cultures. The experiment described above which included all 30 isolates in the same trial substantiated the earlier preliminary investigations. Table 1 presents the disease ratings of the isolates together with their cultural types. Table 2 includes a comparison of parent and variant cultures on a pathogenicity basis.

All isolates and variants of *F. graminearum* proved to be pathogenic, ranging from 5.0 to 0.7 in disease rating. Isolates of cultural Type I had disease ratings of 2.5 and over. On the other hand the cultures of Type II were less virulent, varying from 2.0 to 0.7 in their disease ratings, and

when compared with their parent cultures were significantly less parasitic as shown in figure 3 and table 2. It is of interest to note that the pionnotal variant of 4A of this species was relatively pathogenic with a rating of 2.0, whereas the variant 6A was parasitic only to the extent of 0.7. Taxonomically, variant 4A remained as *F. graminearum* whereas 6A became *F. sublunatum* var. *elongatum*.

As in *F. graminearum* all isolates and variants of *F. culmorum* were pathogenic, but again there was great variation among disease ratings of the cultures, ranging from 4.5 to 0.1. Comparison of the parent Type I cultures with their Type II pionnotal variants again demonstrated the weak pathogenicity of the latter type. (See figure 3 and table 2.) The pionnotal variants which were reclassified taxonomically were all pathogenic to a slight extent as follows: *F. culmorum* var. *cereale*, 1.3; *F. sambucinum*, 0.8; *F. flocciferum*, 0.1; and *F. reticulatum* f.1, 0.1.

Both isolates of *F. equiseti* proved to be slightly pathogenic on wheat seedlings. With this species there was no pre-emergence killing or seedling blight. The disease when present was manifested only as brown lesions on the basal leaf sheaths. The pionnotal variant was slightly pathogenic, but, as in the other species, was less so than the parent form.

Neither isolate of *F. scirpi* showed any evidence of parasitism on wheat seedlings. Strains of *F. scirpi* v. *acuminatum* (Ell. et Ev.) have been proved mildly parasitic by Sprague (18), as have the varieties *F. scirpi* v. *pallens* and *F. scirpi* v. *nigrans* by Bennett (1). The absence of parasitism on seedlings does not rule out the possibility of the cultures herein tested being pathogenic to some degree upon older plants.

Three cultures of *F. sambucinum* obtained from nature likewise caused no disease on wheat seedlings. However, a culture of this species that was obtained as a variant from *F. culmorum*, 7B, showed a low order of pathogenicity, 0.8.

To represent the section Roseum, an isolate of *F. avenaceum* from peas from Montana was tested along with the other species. The pionnotal variant again proved to be less pathogenic than the parent type, having a disease rating of 0.4 as compared to 1.0 for the parent.

Summarizing the data obtained from the pathogenicity studies, the following things become apparent: (1) all isolates of all species except *F. scirpi* and *F. sambucinum* were pathogenic to some extent on wheat seedlings; (2) a culture of *F. sambucinum* that arose as a variant from *F. culmorum* was slightly virulent; (3) the variants of cultural Type II were significantly less parasitic than their parental type in all cases; (4) cultures of *F. sublunatum* var. *elongatum*, *F. flocciferum*, and *F. reticulatum* f.1 were slightly virulent, which constitutes, as far as is known, the first report of their parasitism on cereals; and (5) within one species, *F. graminearum*, was exhibited a range of pathogenicity similar to that of the whole group of species and sections.

These investigations with numerous isolates of the cereal Fusaria, sections Discolor, Roseum, and Gibbosum, disclosed that there were two general cultural types into which all could be placed. The first type was that having an abundance of aerial mycelium with conidia borne usually in sporodochia but occasionally directly in the mycelium (Type I). Cultures with appressed mycelium covered with conidia borne in pionnotes constituted the second type (Type II). Though the former was the most common type, evidence was obtained that the latter type did occur in nature.

Each of the six species studied yielded, upon subculturing with single conidia as inocula, variants of Type II, all of which remained stable in subsequent generations of single-spore culture. Only by continual mass transfer of the original types and the subsequent variants was it possible to obtain reversion of the Type II variant to the parent form. Such apparent reversion is interpreted as a segregation of nuclei that were present from the outset and perpetuated by mass transfer.

When these variants were classified without knowledge of their parent type, many of them fell into species different from those of their parents and some even into different sections. Two of the variants could not be placed in the existing system. Such evidence as this weakens the usefulness of the Wollenweber and Reinking (21) system. Segregation of the cereal Fusaria into distinct morphological units or species becomes difficult when a variant of one species falls into another of the previously described species. New variants may be encountered, as was the case in this work, that may not fit into the existing system and will require new binomials. If these display pathogenicity, as many have thus far, the Fusarium root rot of cereals will have to be considered as caused by an ever-increasing number of *Fusarium* species.

Neither can separation of the sections or species within the sections on a basis of degree of pathogenicity be consistently workable. Members of all show parasitism to some degree on wheat seedlings. Within single species such as *Fusarium graminearum* and *F. culmorum* a range of pathogenicity is exhibited similar to that of the entire groups of species. Johnston and Greaney (12) reported field isolates of *F. culmorum* ranging from very virulent to some that were less pathogenic than *F. equiseti* and *F. avenaceum*.

In view of two facts: first, that members of these sections are difficult to distinguish on the basis of their pathogenicity since single species may embrace the range of pathogenicity of the whole group; and second, that variants of one species may fall into other species or even into other sections on a morphological basis, there would appear to be a close relationship between the three sections and the species comprising them.

How then may the plant pathologist consider this aggregate of Fusarium species, varieties, and forms which cause the cereal root rots? In

the literature, reference is often made merely to *Fusarium* spp. causing cereal root rots. Snyder and Hansen (17) have placed all the species, forms, and varieties of these three sections into one species, *Fusarium roseum* (Lk) Sny. and Hans., designating the forma *cerealis* for all isolates proven pathogenic on cereals.

It should be noted that the writer throughout the text refers to the isolates with definite binomials yet suggests that the relationships were so close that a grouping seems warranted. At the outset, for convenience in handling, each field isolate was identified as closely as possible by reference to *Die Fusarien* (21). As they accumulated, the author was able to recognize certain groups of isolates as fitting into particular species. Other isolates seemed to bridge the gaps between species and varieties within the three sections and were arbitrarily given the name of the species which they most closely resembled. Because many of the variants of a recognized species fell into other recognized species, a definite interrelationship was indicated.

SUMMARY

In a study of the cereal root rot complex in California, isolates of Fusaria were commonly encountered. Because of their evident importance in the root-rot disease, an investigation of the variation, pathogenicity, and taxonomic relationships was undertaken.

Each culture was initiated in pure culture by isolating 20 single conidia directly from the first mycelium advancing from diseased host tissue. These isolates were identified by reference to Wollenweber and Reinking's *Die Fusarien*. Some could be keyed with ease to definite species but others bridged the gaps between species and sections and were arbitrarily given the binomial they most closely resembled. Isolates of *F. graminearum*, *F. culmorum*, *F. sambucinum*, *F. equiseti*, *F. scirpi*, and *F. arenaceum*, representing the sections Discolor, Gibbosum, and Roseum, were included in the studies.

All isolates of all species could be placed in one of two major types, I and II, based on their cultural characters. Type I cultures had abundant aerial mycelium, and the conidia were in sporodochia. This was the type usually found in nature. Type II cultures were appressed and slimy due to the production of numerous conidia in pionnotes. In two cases pionnotal type II isolates were obtained directly from nature, but usually they occurred as culture variants.

All isolates were propagated through 3 to 4 generations by the single-spore technique of transfer. Type I cultures when analyzed by this means frequently yielded variants of Type II. The directional change from Type I to II was observed in all species studied. No change took place in cultures less than 3 weeks old.

Type II variants obtained during single-spore analysis in no case (278 tested) reverted to Type I. Only when employing mass transfer tech-

nique was a Type II culture obtained that subsequently reverted to Type I. This was interpreted as a segregating out of Type I nuclei that had been perpetuated from the outset by mass transfer.

Many variants, when compared from a taxonomic standpoint with their parent cultures, could no longer be considered as the same species nor occasionally even to be in the same section as their parents. A variant of *F. graminearum* had to be reclassified as *F. sublunatum* var. *elongatum*, Three variants obtained from *F. culmorum* were *F. reticulatum*, f.1, *F. sambucinum*, and *F. flocciferum*. A variant of *F. equiseti* could not be classified with any existing species. A crossing of sectional lines from Gibbosum to Roseum was necessitated by a variant of *F. scirpi*.

The pathogenicity of each isolate and variant was determined on wheat seedlings using greenhouse pot tests. All isolates of all species except *F. scirpi* and *F. sambucinum* were pathogenic to some extent. A culture of *F. sambucinum* that arose as a variant from *F. culmorum* was slightly virulent. The variants of cultural Type II were significantly less parasitic than their parental types in all cases. Within a single species such as *F. graminearum* or *F. culmorum*, there was exhibited a range of pathogenicity similar to that of the whole group of species and sections.

It is felt that the evidence presented demonstrates the interrelationship of the Fusarium isolates of the sections Gibbosum, Roseum, and Discolor, both on a taxonomic and a pathogenic basis; and further it tends to substantiate the grouping of all these into one species as advocated by Snyder and Hansen.

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TRANSMISSION OF THE MILD STREAK VIRUS
OF BLACK RASPBERRY[1]

N. L. HORN AND M. W. WOODS[2]

Since mild streak of black raspberry was first described by Dodge and Wilcox (8), many workers have considered this disease to be caused by a virus (1, 4, 7, 8, 12, 13, 14, 15, 16, 17, 18), although experimental evidence of transmission was not presented. Bennett (1) attempted to transmit mild streak with several species of insects but his tests were all negative. In view of the economic importance of mild streak in many black raspberry growing districts, including certain plant producing areas, it was highly desirable to secure definite proof of the experimental transmission of the disease under controlled conditions. The present paper reports experimental transmission of mild streak in black raspberry by cane grafting and by means of dodder.

MATERIALS AND METHODS

Healthy black raspberry (*Rubus occidentalis* L.) layers were obtained from three sources: (1) the commercial variety Logan from an isolated black raspberry patch relatively free from mild streak near Keedysville, Maryland; (2) a hybrid, Cumberland × Evans (referred to hereafter as hybrid plants), from an isolated patch at the University of Maryland farm at College Park, Maryland; (3) the commercial variety Dundee from an isolated patch in a home garden near College Park, Maryland. Diseased Cumberland black raspberry plants were obtained from naturally infected plantings in Western Maryland. In certain instances detached canes of both healthy and diseased field-grown plants from the same locality were used in graft transmission tests.

Grafts between healthy and diseased plants were made by excising tissues of the canes down to the xylem and binding them securely together with Florotape. The usual union extended about 5 to 8 cm. longitudinally along the cane. The test plants were defoliated to increase movement of virus from the source plants; as Hildebrand (9) has shown, creation of a carbohydrate deficit promotes translocation of virus toward deficient areas.

"Dodder grafts" were made by a simplification of Cochran's technique (2, 3) utilizing *Cuscuta subinclusa* D. & H. The test plants in "dodder grafting" experiments were defoliated in the same way, and for the same reason, as in the approach grafts.

Aphids (*Amphorophora sensoriata* Mason) were collected from field-grown healthy and diseased Logan plants in Western Maryland. Sweepings for other insects were also made in a heavily infected Logan patch in the same area. The insects so obtained were identified by Dr. P. W. Oman[3] as leaf hoppers (*Deltocephalus flavicosta* Stal.) and spittle bugs (*Philaenus leucophthalmus* L.).

Throughout all experiments the cane streaking symptom was used as an indication of transmission. Leaf-hooking was not a reliable indication of mild streak in the greenhouse, and the vein-clearing symptom (Fig. 1, A) appears inconsistently on infected plants. The fruit symptom could not be used because berries are produced only during a short period in the greenhouse (Fig. 2, C).

<div align="center">RESULTS</div>

Graft transmission. In a preliminary experiment (June 22, 1946), 10 healthy Logan plants were approach-grafted to 10 diseased Cumberland plants in the greenhouse. Controls included 10 approach grafts between apparently healthy plants of either Logan or Dundee. Both of these varieties are known to be susceptible to mild streak (16). About two months after grafting, cane streaking began to appear on five of the test plants, and four of the Logan control. At the end of the summer the grafts were broken, and all plants were heavily pruned and then placed out of doors from September 21, 1946, to January 20, 1947. Two months after the plants had been returned to the greenhouse, three more of the test plants showed streaking on the new vigorously growing shoots. All plants were observed until May 28, 1947, but cane streaking did not appear on any of the remaining plants. Several other plants from this supposedly healthy stock, but which were not used in the experiment, also developed mild streak at this time. It is evident, therefore, that some residual infection was present at the beginning of the experiment.

Thirteen healthy hybrid plants, three healthy Dundee plants, and four healthy Logan plants, all of which were defoliated except for a few terminal leaves were approach-grafted to 20 diseased Cumberland plants. Controls consisted of the same number of plants of each of the same varieties treated similarly, and approach-grafted to 20 healthy hybrid plants. The results are given in table 1. All plants were held until November 25, 1947. None of the remaining plants developed cane symptoms. Of those that became infected, streaking appeared first on some plants on the new lateral growth below the union, on other plants first at the base of canes other than the grafted ones, and on still others on both grafted and non-grafted canes of the same plant.

In another experiment, transmission was obtained by approach-grafting 10 healthy Dundee plants, which were defoliated except for a few terminal leaves, to 10 diseased plants. Controls consisted of 10 approach grafts

between healthy Dundee plants treated similarly. All grafts were made
on October 10, 1947. No symptoms were observed on any of the test plants
by December 15, 1947, at which time all grafts were broken, each plant was
pruned heavily, and then all plants were subjected to dormant conditions
(winter temperatures) until February 2, 1948. New vigorous growth was
resumed and cane streaking was observed on one test plant 68 days later,
and by May 11, 1948, a total of 9 of the 10 test plants showed cane symp-
toms. Streaking was not observed on any of the control plants.

Some field-grown plants were observed in which the canes were heavily
streaked, while in others streaking was moderate, and in still others so

TABLE 1.—*Transmission of mild streak in black raspberry by grafting*

Graft number[a]	Date graft was made	Number of days before streaking appeared on test plants[b]
1	March 26, 1947	39
2	do	77
3	do	77
4	do	39
5	do	77
6	do	77
7	do	103
8	do	77
9	do	77
10	do	39
11	July 5, 1947	90
12	do	75
13	do	no symptoms
14	do	do
15	do	do
16	do	do
17	do	75
18	do	75
19	do	90
20	do	75

[a] Plants 1–13 were healthy hybrids, 14–16 were healthy Dundee, and 17–20 were
healthy Logan. All were grafted to diseased Cumberland plants.
[b] Controls, consisting of 20 healthy plants grafted to 20 healthy plants, remained
negative.

faint as to be barely discernible. Five cuttings of the current-season canes
from plants of each of these symptom types were approach-grafted to
healthy Logan plants in the greenhouse. Five cuttings from healthy Logan
plants in the field were approach-grafted to five healthy Logan plants in
the greenhouse as controls. All grafts were made on August 2, 1946. On
December 3, 1946, the plants were pruned heavily and placed out-of-doors
where they remained for the duration of the experiment. On June 21, 1947,
the plants were rated as to the severity of streaking (Table 2).

Dodder transmission. Thirty diseased plants were "dodder-grafted"
(3) to 30 healthy plants in the greenhouse with *Cuscuta subinclusa.* The
test plants were defoliated except for a few terminal leaves, and only the
stem area required for grafting was defoliated on source plants. Thirty

TABLE 2.—*Graft transmission of mild streak, from field cuttings of Logan black raspberry differing in severity of symptoms, to healthy plants of the same variety*

Severity of streak in field plants from which affected canes were taken	Number of plants infected[a] and severity of symptoms			
	Mild	Medium	Pronounced	Apparently healthy
Mild	1	2	1	1
Medium	1	1	1	2
Pronounced	1	0	2	2
Controls	0	0	0	5

[a] Five plants were grafted in each group.

healthy plants "dodder-grafted" to 30 healthy plants in a similar manner were used as controls. The results are shown in table 3. The first indica-

TABLE 3.—*Transmission of mild virus in blackberry by "dodder grafting"*

Graft number	Varieties grafted[a]		Number of days before appearance of cane symptoms[b]
	Test plant	Source plant	
1	Cumberland × Evans	Logan	67
2	do	do	no symptoms
3	do	do	67
4	do	do	no symptoms
5	do	do	84
6	do	do	no symptoms
7	do	do	do
8	do	do	67
9	do	do	no symptoms
10	do	do	do
11	Logan	do	do
12	do	do	72
13	do	do	no symptoms
14	do	do	do
15	Dundee	do	do
16	do	do	do
17	do	do	do
18	Cumberland × Evans	Cumberland	do
19	do	do	do
20	do	do	do
21	do	do	do
22	do	do	do
23	do	do	do
24	do	do	do
25	do	do	do
26	do	do	do
27	do	do	54
28	do	do	54
29	do	do	54
30	do	do	no symptoms

[a] Grafts 1–10 were made on April 11, 1947; 11–20 on July 7, 1947, and 21–30 on August 28, 1947. All plants were observed for five months before discarding.

[b] Dodder grafts between 30 pairs of healthy plants of the same variety as the test plants were used as controls which remained negative throughout the experiment.

tion of transmission was a hooking of the younger leaves (Fig. 1, C).
Several weeks later slight cane lesions developed on the new basal growth.
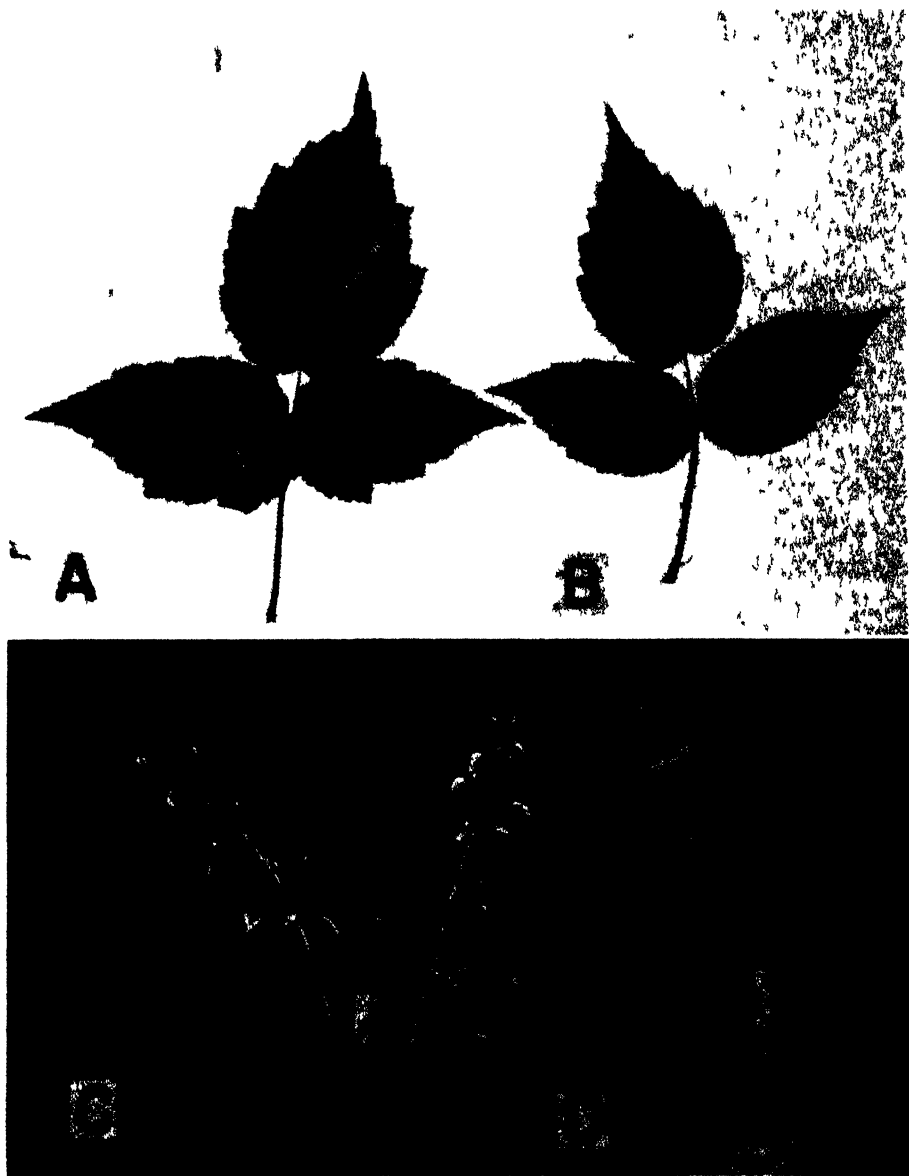
FIG. 1. A. A leaf taken from a diseased hybrid (Cumberland × Evans) plant show-
ing the vein clearing symptom. B. A leaf taken from a healthy hybrid plant. C.
Hooked leaves on a diseased plant of the Cumberland variety. D. Normal, flat leaves
on a healthy hybrid plant.

Further development consisted of an increase in the amount of these cane
streakings (Fig. 2, A).

Since the canes involved in "dodder-graft ng" had to be bound tightly,
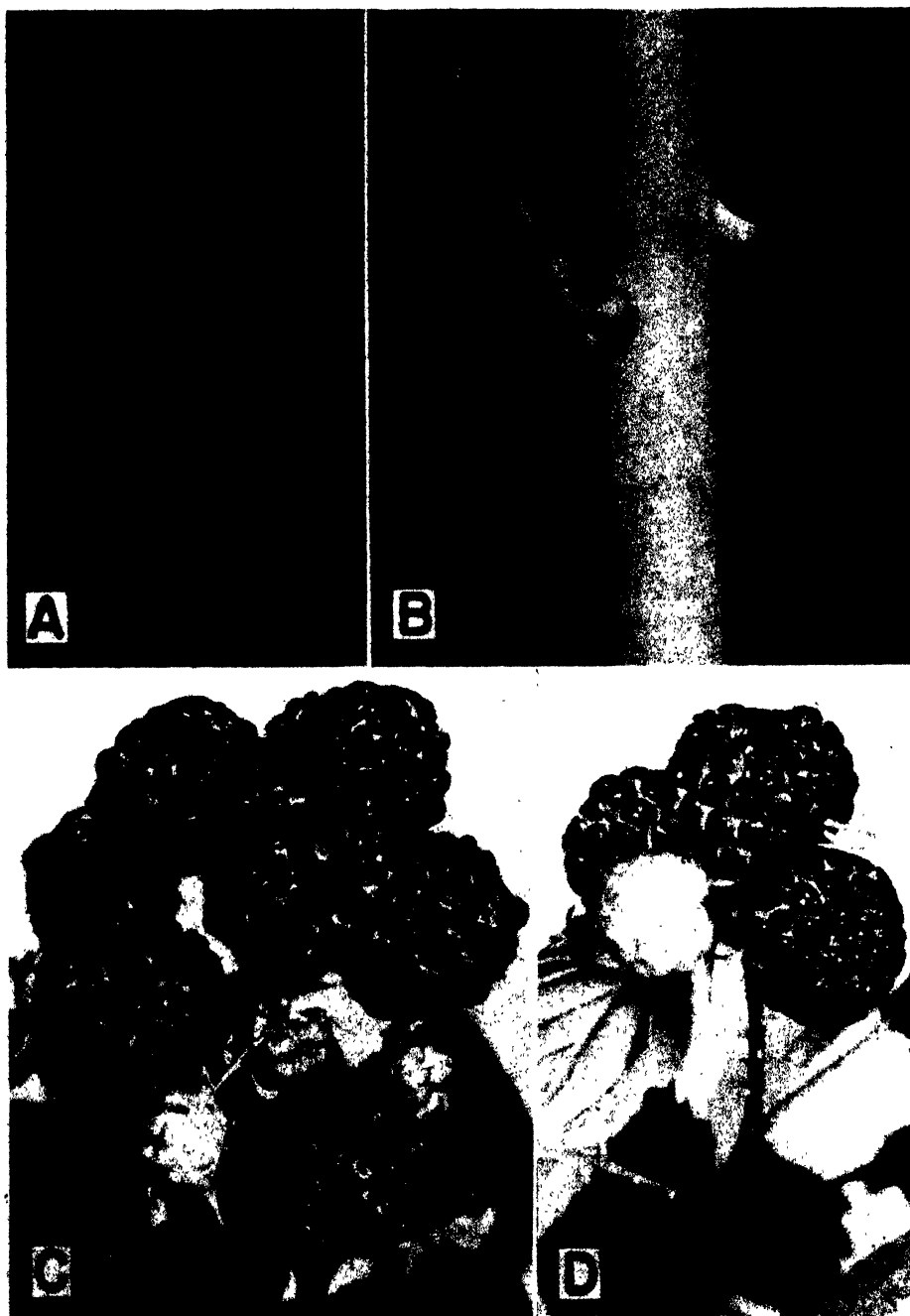
FIG. 2. A. A portion of a cane (Cumberland variety) severely infected with mild streak. B. A portion of a healthy cane (Cumberland × Evans), showing the clean, white appearance of the bloom. C. Berries from a diseased Cumberland plant, showing the over-all dull appearance of the fruit and the sunken drupelets, approximately 1×. D. Berries from a healthy Logan plant, showing the fullness of each drupelet and the healthy shiny appearance of the fruit in general, approximately 3/4×.

some injury may have occurred at the points of binding so that an unintentional union could have been made between the test and source plants. In order to test this possibility of unintentional grafting, 10 healthy plants were attached to 10 diseased plants in the manner described for "dodder-grafting" but void of dodder. There was no evidence of union between any of the canes at the end of three months, and no symptoms occurred on any of the test plants within eight months.

Mechanical transmission. Three healthy Logan, three healthy Dundee, and four healthy hybrid plants were inoculated mechanically with freshly prepared sap from leaves of diseased plants. The expressed sap was obtained at greenhouse temperature (approximately 85° F. by crushing freshly harvested leaves from diseased plants in a sterilized mortar. A 6-inch pot label, wrapped at the blunt end with sterile gauze, was used to inoculate the plants. Carborundum[6] was mixed with the inoculum, and 10 leaves of each test plant were inoculated at random. Ten healthy plants of the same varieties were similarly inoculated as controls, with expressed sap prepared in the same manner from leaves of healthy plants. Inoculations were made on June 20, 1947, and final observations were made on November 7, 1947. No symptoms were apparent at any time on either the test or control plants.

Insect transmission. Stem aphids (*Amphorophora sensoriata*) were collected on June 20, 1946, from a planting of Cumberland black raspberries which contained both healthy and diseased plants. In the greenhouse, seven hours later, aphids which had been feeding on apparently healthy plants in the field were caged on healthy Dundee and Logan plants and diseased Cumberland plants. Aphids which had been feeding on diseased plants in the field were caged on healthy Dundee and Logan plants. All insects began to feed within an hour after transfer and were permitted to feed for five days. Sub-colonies were made until a total of 44 healthy Dundee and Logan plants had been exposed to insects which had been feeding on diseased plants, and 21 healthy Dundee and Logan plants had been exposed to insects which had been feeding on healthy plants. Attached tip layers were rooted from all the test plants at the time the insects were caged on them. Both layers and parent plants were held through the following spring of 1947, but no symptoms were observed on any of the plants.

Insect sweepings were made in a Logan black raspberry patch at Keedysville, Maryland, on June 27, 1947. This planting was known to be 90 per cent infected with mild streak disease. Within five hours the insects were transported to the greenhouse where they were treated with sufficient chloroform vapor to render them inactive for 10 minutes in order to segregate them into generic groups for counting. The insects used were leafhoppers (*Deltocephalus flavicosta* Stal.) and spittle bugs (*Philaenus leucophthalmus*

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[6] Grade No. 600, Manufactured by the Carborundum Company, Niagara Falls, New York.

L.).   Twenty Logan and Dundee plants growing in the greenhouse under
one large cage (mosquito netting) were exposed to 62 of the leafhoppers
and 95 of the spittle bugs.   Controls consisted of 20 Dundee and Logan
plants under a similar cage from which insects were excluded.   No symptoms appeared on any of the plants by November 15, 1947.

<div align="center">DISCUSSION</div>

The mild streak disease of black raspberry has been described repeatedly
and, although evidence of experimental transmission has been lacking, the
disease has usually been assumed to be of virus nature.   A preliminary
report (10), however, presented evidence of graft transmission.   In the
present study the disease was graft-transmitted from infected black raspberry plants to healthy black raspberry plants in 35 of 45 attempts.   The
results of an early experiment in which some of the controls were infected
are not included in the figures mentioned, since it seems likely that the
supposedly healthy controls used in this experiment were already infected
by natural means before the experiment was begun.   Transmission was obtained by "dodder grafting" with *Cuscuta subinclusa* in 8 of 30 attempts.
In view of the type of symptoms occurring in diseased plants, together with
the demonstration of graft transmissibility, it is now considered justifiable
to regard mild streak as definitely of virus nature.

The wide variation in severity of symptoms in the field, and in plants
artificially inoculated, has suggested the possibility of strains of the mild
streak virus.   The data obtained, however, showed that the degree of severity of cane streaking was not maintained when the virus was introduced
into healthy plants by grafting.   A more extensive study along this line
should be undertaken.

On the basis of field observations, Rankin (13), Slate et al. (15), Cooley
(4, 5, 6), and Jeffers and Woods (11) suggested that the mild streak virus
may be transmitted by an insect.   Bennett (1) found that *Amphorophora
sensoriata* does not transmit the disease in Michigan, and Cooley (6) suggested that the vector is not an aphid.   In the present study an aphid
(*A. sensoriata*), a leafhopper (*Deltocephalus flavicosta*), and the spittle
bug (*Philaenus leucophthalmus*) were tested as possible vectors of mild
streak, but in none of the tests did these insects transmit the disease.   An
extensive program involving field collections and testing of insects at short
intervals throughout the spring and summer should be conducted with the
above mentioned insects before finally excluding them from a list of possible
vectors.

The expressed sap from the leaves of black raspberry plants is readily
oxidized at temperatures of 70° F. and higher (1).   This might explain
failure to obtain mechanical transmission in the present investigation, although other factors may also be involved.

<div align="center">SUMMARY</div>

The mild streak disease was found to be graft-transmissible from dis-

eased Cumberland and Logan black raspberry plants to healthy Logan, Dundee, and hybrid plants (Cumberland × Evans) in 43 of 55 attempts.

Transmission was obtained in 8 of 30 attempts by "dodder grafting" with *Cuscuta subinclusa* from diseased to healthy black raspberry plants.

The virus nature of the mild streak disease of black raspberry is now considered to be established.

Existence of strains of the virus was not demonstrated since variations in the degree of severity of cane symptoms occurring on the variety Logan in the field were not transmitted consistently by grafting to healthy Logan plants in the greenhouse. *Amphorophora sensoriata* Mason, *Deltocephalus flavicosta* Stal., and *Philaenus leucophthalmus* L. did not transmit the virus to black raspberry plants under the experimental conditions employed.

No transmission was obtained by mechanical inoculation of healthy black raspberry plants with freshly expressed sap from the leaves of mild streak diseased black raspberry plants.

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THE INFLUENCE OF NITROGEN SOURCE ON THE DEVELOPMENT OF STEM RUST OF WHEAT[1]

J . M . D A L Y [2]

INTRODUCTION

It is well established that environmental conditions may have a pronounced effect on the stem rust reaction of wheat seedlings (1, 11). With certain combinations of host and physiologic race of rust, increasing temperatures and light intensities will shift the infection type from a resistant to a susceptible class. The present work was undertaken to determine whether shifts in infection type could be induced through changes in nutrient balance and whether such shifts would explain some of the interactions between host and parasite.

Throughout this study, the wheat varieties Mindum (C. I. 5296), Marquis (C. I. 3641), and Thatcher (C. I. 10003) were inoculated with physiologic race 56 of *Puccinia graminis tritici* because these varieties are resistant, susceptible, and mesothetic, respectively, to this race. The method of inoculation and criteria of rust infection were those of Stakman, Levine, and Loegering (19). Resistant reactions are indicated by infection types 0, 1, and 2, the susceptible class includes infection types 3 and 4, while the mesothetic reaction is designated X. The individual infection types are further designated by plus or minus signs indicating that the infection type approaches the maximum or minimum for the type. This is based on the amount of sporulation, necrosis, or chlorosis.

NUTRITIONAL STUDIES IN SAND CULTURE

Plants of the three varieties were grown in washed quartz sand contained in glazed crocks of 2000-cc. capacity. A 500-cc. Erlenmeyer flask, serving as a reservoir, was attached by rubber tubing to an outlet at the base of each crock. By alternate inversion of the flask above and below the level of the crock, nutrient solutions could be rapidly drained from the sand at intervals during the day, thus promoting a favorable oxygen tension for root development. This arrangement also permitted a ready change of nutrient every five days.

Each crock accommodated ten plants of each variety, and three replicates were used for each treatment. Nutrients were applied after the primary leaves had broken through the coleoptile. The major elements were supplied as three-salt solutions of equal osmotic pressure, as outlined by Livingston (12); the nature of the three salts composing a particular nutrient solution

was governed by the balance of ions desired for the treatment. Nitrogen in all cases was supplied as nitrate. Minor elements were furnished as their sulfates; iron, 2 ppm; boron, 0.5 ppm; copper, manganese, and zinc, 0.08 ppm. The wide diversity in composition of the 38 nutrient solutions tested

TABLE 1.—*Composition of 38 three-salt solutions and the rust infection types on Mindum, Marquis, and Thatcher wheats inoculated with race 56 of Puccinia graminis tritici*

Solution	K	P	Ca	N	Mg	S	Rust infection types on		
			P.p.m.				Mindum	Marquis	Thatcher
1	95	75	200	140	305	395	1 − to 1 +	3 − to 3	1 = to 1 +
2	85	65	435	305	105	140	0; to 1 ++	3 − to 3 +	1 − to 1 +ᵃ
3	200	160	110	80	325	425	0; to 1	3 − to 3	1 − to 1ᵃ
4	180	140	285	200	175	230	0; to 1	3 − to 3	1 − to 1 ++
5	155	120	440	310	50	70	0; to 1	3 − to 3 +	1 to 3 = to 3 +
6	260	205	275	195	110	145	1 − to 1 +	3 − to 3 ++	1 to 3 = to 3 +
7	470	370	120	85	120	160	1 − to 1 +	3 − to 3 +	1 = to 1 +
8	450	355	190	135	60	75	0; to 1	3 = to 3 +	1 = to 1 +
9	75	600	155	110	235	60	0; to 1 ++	3 = to 4 +	3 = to 3 +
10	75	230	375	260	90	60	0; to 1	3 − to 3 +	3 − to 3 +
11	150	590	90	55	230	120	0; to 1 ++	3 to 4 +	3 = to 4 −
12	140	340	215	150	130	115	0; to 1 +	3 − to 4	3 = to 3 ++
13	140	110	365	250	45	115	0; to 1 ++	3 to 4 +	3 − to 3 ++
14	220	250	230	160	90	180	0; to 1 +	3 to 4 −	3 = to 3 +
15	365	230	90	50	90	300	0; to 1 ++	3 − to 4 +	3 = to 3 +
16	370	120	150	105	45	300	0; to 1 ++	3 to 4 −	3 = to 3 +
17	100	330	210	35	320	425	1 − to 1 ++	3 to 4 +	3 = to 3 +
18	230	170	110	80	325	435	0; to 1	3 − to 4 +	3 − to 3 +
19	175	420	270	65	160	215	1 to 1 ++	3 − to 4	3 = to 3
20	260	410	270	95	110	145	1 − to 1 ++	3 to 4 −	3 = to 3 +
21	490	150	95	175	115	155	0; to 1 +	3 − to 4 +	3 = to 3
22	430	280	180	160	55	75	0; to 1 +	3 to 4 +	3 = to 3
23	140	200	125	265	210	55	0; to 1	3 − to 3	3 = to 3 −
24	135	575	360	100	85	60	0; to 1 +	3 − to 3 +	3 = to 3 −
25	275	110	70	260	110	110	0; to 1 +	3 = to 3	3 − to 3
26 ·	275	350	220	155	130	110	0; to 1	3 − to 3	3 = to 3 −
27	295	600	380	55	45	120	0; to 1	3 − to 4 −	3 − to 3 +
28	430	350	220	105	85	180	0; to 1 +	3 − to 4 −	3 = to 3
29	750	120	75	100	90	300	0; to 1	3 − to 3	3 − to 3 +
30	760	240	155	54	45	310	0; to 1	3 = to 3 +	3 = to 3 +
31	80	650	160	20	250	130	0; to 1	3 = to 3 ++	3 = to 3 −
32	90	250	410	30	100	330	0; to 1 =	3 = to 3 −	3 = to 3 −
33	150	650	65	55	250	65	0; to 1 +	3 = to 4 −	3 − to 3
34	165	415	260	60	160	210	0; to 1 +	3 = to 3 +	3 = to 3 +
35	185	155	475	65	60	380	0; to 1	3 − to 3 +	3 − to 3 +
36	260	295	270	100	115	230	0; to 1	3 = to 3 −	3 − to 3
37	395	270	90	155	110	75	0; to 1 +	3 − to 3 +	3 − to 3
38	400	145	175	155	55	145	0; to 1	3 − to 3 +	3 = to 3 −

ᵃ Rust reactions based on only two replicates.

is shown in table 1, in which concentration of the six major elements is given, for convenience, in parts per million.

The plants were grown at temperatures above 80° F., and inoculated with rust 20 to 25 days after planting. Notes on rust development were obtained 13 or 14 days after inoculation. Because of limitation in space, only eight solutions were tested in a single experiment.

Plant vigor was relatively uniform and normal. Solutions 9, 14, 36, 37,

and 38 produced slightly chlorotic plants. The concentrations of calcium and phosphorus in solutions 24 and 27 caused a slight tip-burn.

Despite the variation in composition of nutrient solutions there was only slight evidence that rust development was affected (Table 1). Mindum was resistant and Marquis was susceptible in all cases. Thatcher was moderately susceptible (infection type 3) except during a period of low light intensity in late winter (solutions 1 through 8). In solutions 5 and 6, susceptible reactions developed on some of the plants, although plants in the remainder of the six treatments tested at the time were resistant. Solutions 5 and 6 contained relatively high proportions of nitrogen, but nitrogen was not the only factor involved since Thatcher grown in solution 2, higher nitrogen but lower potassium and phosphorus, was resistant.

These results suggested that light and possibly temperature might tend to mask the effects of nutrition. Since only nitrate had been used as a nitrogen source in sand culture and because of the differential assimilation of ammonium and nitrate nitrogen at various pH levels (21), combinations of these two factors, in conjunction with temperature, were tested.

THE INFLUENCE OF NITROGEN AND TEMPERATURE ON RUST DEVELOPMENT

Five plants each of Thatcher and Mindum wheat were grown in a mixture of 3 parts sand and 2 parts greenhouse soil in a single 4-in. pot. After the seedlings had emerged, 100 cc. of solution containing 400 ppm. of nitrogen as one of the following salts was applied every other day: potassium nitrate, KNO_3; calcium nitrate, $Ca(NO_3)_2$ $4H_2O$; ammonium nitrate, $NH_4 NO_3$; ammonium sulfate, $(NH_4)_2SO_4$. The check consisted of similar applications of a solution of 0.5 gm. of potassium phosphate (KH_2PO_4), magnesium sulfate $(MgSO_4)$, and calcium nitrate $(Ca(NO_3)_2$ $4H_2O)$. Three replicates of each treatment were maintained at pH 8.0–9.5 and three at pH 4.0–4.5. The plants were placed at different temperatures on the first day nitrogen was supplied. Six days later they were inoculated with race 56 of *Puccinia graminis tritici*, and final rust observations were made 15 to 19 days after inoculation.

Rust reaction at low temperature. Plants supplied nitrogen differed only slightly in vigor. There was a slight tendency for nitrate-treated plants to tiller more than ammonium-treated plants in the first experiment, but in later experiments no differences were noted. The check plants receiving low nitrogen were distinctly stunted and chlorotic. The rust reaction of Mindum was not altered by nitrogen source; all plants were resistant (0; to 1 infection types), although the check plants had fewer pustules.

The check plants of Thatcher wheat, and those supplied ammonium sulfate, were resistant to rust (0; to 1 infection types). Plants supplied potassium nitrate were mesothetic (X to X ++ infection types) approaching very closely to complete susceptibility (Table 2 and Fig. 1). Calcium nitrate
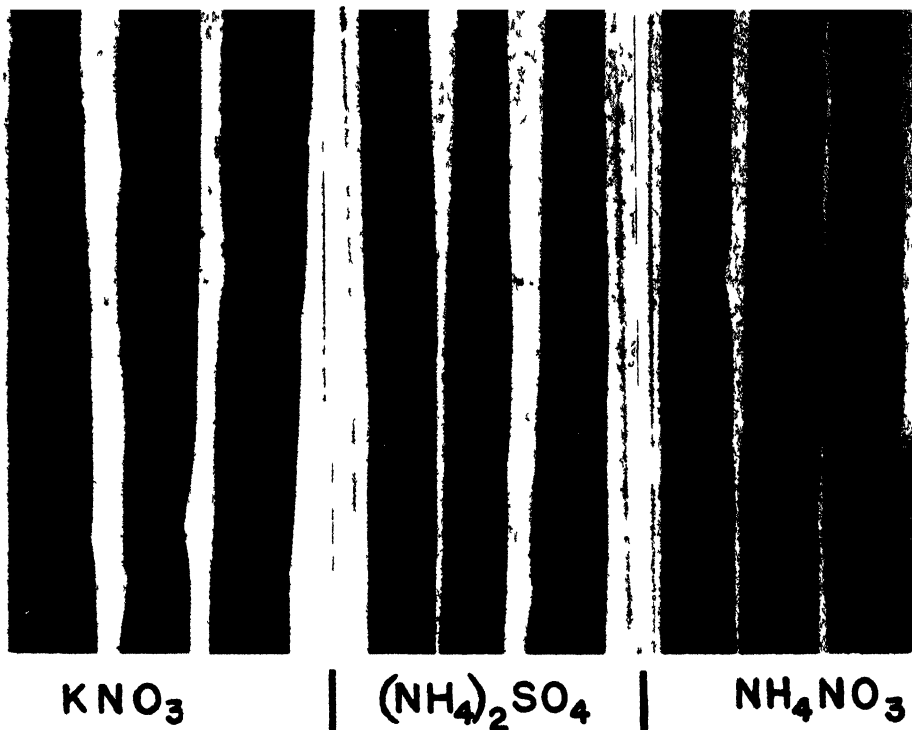
FIG. 1. Infection types produced by race 56 of *Puccinia graminis tritici* on Thatcher wheat supplied three different nitrogenous salts KNO_3, x to x++ infection type $(NH_4)_2SO_4$, 0, to 1 infection types   The center leaf has two pustules of powdery mildew. NH_4NO_3, x   to x infection types

treated plants were slightly more resistant (X – to X ++ infection types) The application of 400 ppm of nitrogen as ammonium nitrate (200 ppm of NH_4^+ and 200 ppm NO_3) resulted in plants intermediate in reaction between ammonium and nitrate plants   Some leaves were completely resistant and some were mesothetic, but with evident resistance   Since pH did not alter rust reaction, the results of all pH levels for two experiments are grouped in table 2

In later tests, comparisons were made of the nitrogenous constituents of

TABLE 2—*The influence of nitrogen source on the rust reaction of Mindum and Thatcher wheats inoculated with race 56 of Puccinia graminis tritici and grown at 65° – 75° F*

Treatment		Rust infection type on ·	
		Mindum	Thatcher
KNO		0; to 1 ++	x + to x ++
Ca(NO₃) 4H₂O		0; to 1 ++	x = to x ++
NH₄NO₃		1 to 1 ++	0; to 1 ++ (22 leaves)
			x = to x + (30 leaves)
(NH₄)₂SO₄		0, to 1 ++	0; to 1 +
Check		0; to 1 +	0; to 1 +

Thatcher and Mindum wheats under these conditions. Ten pots of each variety were supplied with nitrogen salts as outlined above, but no attempt was made to adjust the pH of the sand-soil mixture. Eleven days after first application of the salts, 2 pots of each treatment were removed from the greenhouse bench and inoculated with race 56 of stem rust. Ten days after inoculation the leaves of the inoculated plants began to fleck. In the evening, 30 gm. of leaves of the same age were removed from the remaining 8 noninoculated plants and immediately autoclaved at 5-lb. pressure for 5 min. The leaves were dried at 60° C. and weights were recorded. The leaves were harvested at the time of first visible symptoms of rust infection since it was believed, from other evidence, that the metabolic state of the leaves at this time establishes the ultimate rust reaction. Final rust reaction of inoculated plants was estimated 10 days after flecking.

Total nitrogen was determined by the micro-Kjeldahl method after preliminary reduction with zinc. Soluble reducing substances were de-

TABLE 3.—*Rust reaction and composition of Thatcher and Mindum wheats grown at low temperatures and inoculated with race 56 of Puccinia graminis tritici*

Treatment[a]	Thatcher			Mindum		
	KNO₃	(NH₄)₂SO₄	NH₄NO₃	KNO₃	(NH₄)₂SO₄	NH₄NO₃
Rust reaction	x - x ++	0; - 1	0; - 1 -[c], x - x ++	0; - 1	0; - 1	0, - 1
Dry weight (per cent)	14.2	13.8	14 3	17.0	15 6	15 7
Content[b] of						
Total N	45.8	51.1	51 9	43.5	48 6	50 7
Protein N	37 7	41 6	42.3	36.6	39 9	41 5
Ammonia N	0.14	0.57	0 40	0 10	0 36	0.28
Amide N	0 46	0.79	0.60	0 18	0 26	
Amino N	2 98	4.16	3.64	2.69	3 22	2 95
Total carbohydrate	76.0	66.6	60 4	93 2	85 6	78 4
Reducing substances	8.2	14.8	13.6	9.0	17.0	16.0

a 400 ppm. nitrogen.
b Expressed as mgm. per gm. dry weight.
c Two leaves with resistant reaction.

termined by a modification of the Shaeffer-Somogyi (10) procedure and total soluble carbohydrate by the same method after acid hydrolysis. Protein nitrogen was estimated in the micro-Kjeldahl apparatus on the residue from alcohol extraction of sugars. Amide and ammonia nitrogen were extracted with water and determined by the method of Pucher *et al.* (16). Amino nitrogen in the same extract was determined by the van Slyke procedure.

The results of analysis, and the rust reactions of inoculated plants, are presented in table 3. Thatcher wheat grown with ammonium nitrate did not have so high a degree of resistance as in previous experiments, as only two leaves were resistant. Infection of the remainder of the leaves was of the mesothetic class, but the number of resistant pustules was greater than in plants supplied with potassium nitrate. The analytical results indicate

that none of the leaf fractions examined can be definitely linked with the factors responsible for resistance and susceptibility in this instance. There is no correlation between rust resistance and total or protein nitrogen. Resistant leaves of Thatcher have a higher content of both forms of nitrogen than do the leaves of susceptible plants, but plants supplied ammonium nitrate have still greater amounts of total and proten nitrogen even though the leaves are slightly more resistant. A similar but inverse relationship is found with total soluble carbohydrates.

Soluble nitrogen levels induced by the various treatments form a pattern that follows the degree of resistance of the leaves. Resistant leaves have the highest content of ammonia, amide, and amino nitrogen, while moderately susceptible leaves have approximately 1/4 the ammonia, 1/2 the amide nitrogen, and 2/3 the amount of amino nitrogen. Plants supplied with ammonium nitrate are intermediate with respect to these compounds. However, the same relative differences in soluble nitrogen can be observed in Mindum, but without a corresponding change in rust reaction.

The trends for soluble reducing substances are the same as for soluble nitrogen components, but this may indicate a nonspecificity of the sugar reagents which becomes apparent only when the ratio of amino acids to monosaccharides is low. The lower content of nitrogenous compounds and higher content of sugars in Mindum can be ascribed to a varietal difference in nitrogen metabolism.

Although it is impossible to single out one fraction as responsible for protoplasmic resistance of wheat to stem rust, the possibility remains that the balance of metabolites in the leaves of Thatcher determines the resistance of this wheat to race 56, but that in Mindum wheat another leaf constituent, not revealed by these data, exercises more influence in controlling rust reaction to this race.

Rust reaction at high temperatures. Attempts to grow plants under the same conditions at temperatures between 80° and 90° F. were not successful. The plants grew abnormally fast and lacked the vigor of the plants grown at low temperature. Although rust readings were made, the condition of the plants does not justify serious consideration of the results. Thatcher in all cases was susceptible (infection types 3 to 4), while Mindum was resistant (infection types 0; to 1).

FIELD EXPERIMENTS

Plots of three rows each of Mindum, Marquis, and Thatcher wheat were planted on a light sandy loam. Each plot was eight feet square with 2-ft. alleys between plots. Border rows were inoculated with race 56, to supply inoculum for the natural spread of rust to the plots. Treatments consisted of 200 lb. per acre of calcium nitrate, and 270 lb. per acre of urea, ammonium sulfate, or ammonium nitrate applied at time of planting. Each treatment was replicated three times.

Plots treated with nitrogen were uniformly more vigorous than the

checks. The plants were taller, tillered well, and were darker green than control plants. There was no influence of nitrogen on the percentage or type of rust infection. Marquis was susceptible, averaging 30 per cent infection, while Mindum was resistant.

Thatcher wheat, normally mesothetic in the seedling stage, developed only a trace of stem rust since this variety has a high degree of adult plant resistance. This is perhaps largely due to morphogical characteristics and would tend to mask any change in the protoplasmic resistance.

DISCUSSION

Most investigations concerning nutrition of wheat in relation to the development of stem rust have been made in the field and, as Gassner (2) and Stakman and Aamodt (18) have pointed out, the effects of fertilizers are largely indirect, since photoplasmic resistance, the cellular incompatibility between host and parasite, has not been altered. In general, the addition of nitrogenous compounds delays maturity, increases density of stand, and promotes succulence, conditions conducive to increasing the number of infections but not necessarily to change in infection type.

Nutritional studies on seedlings in the greenhouse have yielded negative results (25), except in instances where experimental conditions have visibly altered the vigor of the host. It is well known that vigor of the host is directly correlated with the development of rust and if the general well-being of the host has been affected, it can not be shown with certainty that the normal protoplasmic factors governing rust resistance have been changed. Instead, secondary factors may be operative. Darley and Hart (1), however, reported that low nutrient levels produced relatively low infection types on Kenya wheat, while high nutrient levels raised the infection types.

The results presented here indicate that failure to find a positive influence of nutrition on rust development may be associated with interactions of temperature, nitrogen source, and the host-parasite combination. These interactions have been investigated in connection with Fusarial diseases by Walker and recently summarized (23). The resistance of Thatcher wheat to race 56 of stem rust is analogous with the "type B" resistance possessed by some cabbage varieties to *Fusarium oxysporum* f *conglutinans*. At high temperatures nitrate nitrogen did not influence rust reaction even though the concentrations ranged from 20 to 305 ppm. At low temperatures, plants supplied 400 ppm. of nitrate nitrogen were moderately susceptible but 200 ppm. of nitrate nitrogen lowered the susceptibility, even though the plants received an additional 200 ppm. of ammonium nitrogen. Plants receiving 400 ppm. nitrogen in the form of the ammonium ion were completely resistant. Mindum wheat corresponds to cabbage varieties with "type A" resistance in that none of the environmental factors investigated caused shifts in rust reaction. Thatcher and race 56 of stem rust appear to be in delicate balance at temperatures between 60° and 75° F., and only at that temperature can the balance be upset by nutrition.

Gassner and his coworkers (3, 4, 5, 6) reported that increasing amounts of nitrogen raised the susceptibility of wheat to stripe and leaf rusts. The varieties most subject to variation were the moderately susceptible or moderately resistant types. Later these workers concluded that susceptibility was correlated with the concentration of protein, but not with soluble nitrogen. Mains, on the other hand, working with several rusts (13), thought that obligate parasitism and resistance to rust were dependent on the availability of carbohydrates. The present analysis of the wheat varieties grown at low temperatures indicates that changes of rust reaction as a result of nitrogen nutrition are accompanied by changes in both carbohydrate and nitrogenous compounds, particularly the individual soluble nitrogen compounds.

These results are in accord with the literature on nitrogen metabolism (15) and it appears impossible to predict, from experiments in which disease resistance has been altered by manipulation of one or two environal conditions, which class of leaf constituents is fundamental to the change in disease expression. For example, protein content of the cell has been shown to be reflected in the respiration rate (8, 9, 17, 26), photosynthetic activity (9), ascorbic acid content (27), organic acids (22, 26), and salt uptake (20). Protein content, in turn, is regulated by available carbohydrate (17, 26), supply of potassium and phosphorus (8, 17), and the presence of "sinks" in other plant parts (24). It is therefore necessary to investigate a wide number of leaf constituents and to consider the influence of interlocking metabolic systems before implicating one process or group of compounds (protein, sugar, ammonia (7), phenols (14)) as the source of rust resistance.

SUMMARY

At temperatures above 80° F., mineral nutrition had little effect on the rust reaction of susceptible (Marquis), resistant (Mindum), or moderately susceptible (Thatcher) wheats. At temperatures between 65° and 75° F., Thatcher wheat was completely resistant to race 56 of *Puccinia graminis tritici* when supplied ammonium nitrogen and mesothetic, but approaching complete susceptibility, when supplied equivalent amounts of nitrate nitrogen. The response of Thatcher treated with ammonium nitrate was between these extremes. Mindum wheat was resistant regardless of nitrogen source.

Resistant and mesothetic leaves of Thatcher and resistant leaves of Mindum were analyzed for total, protein, amide, amino, and ammonia nitrogen, as well as for total soluble carbohydrates and reducing substances. The results are discussed in relation to existing theories of rust resistance in wheat.

Nitrogen source did not influence the rust reaction of Marquis, Mindum, or Thatcher wheat in the field.

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A METHOD FOR THE SELECTIVE STAINING OF VIRUSES IN INFECTED PLANT TISSUES[1]

J . G . B A L D

INTRODUCTION

The method of fixing and staining viruses in infected plant tissues described in this paper was evolved from trials with tobacco mosaic virus in tobacco. This virus produces three kinds of inclusion bodies in the parenchymatous cells of its host plant (11), two of which have been shown by inoculation to contain high concentrations of virus.

The criteria of successful staining demanded of this method applied to tobacco mosaic were (1) that all three types of inclusion body should stain in the same way, and (2) that samples of purified tobacco mosaic virus *in vitro* should react to fixation and staining in much the same way as the inclusion bodies in the tissues. This paper outlines the development of the method, and suggests a staining schedule that has been widely tested on tobacco mosaic and other viruses.

PRELIMINARY STAINING OF PURIFIED VIRJS AND INFECTED TISSUES

Spot tests with purified virus. Spots of purified tobacco mosaic virus were dried on slides at $40°-45°$ C. from drops of a concentrated suspension in water. They were subjected to various treatments designed to expose the ribonucleic acid fraction of the virus (6) to the action of dyes. After the spots were submitted to these treatments, Giemsa stain was applied to them in order to demonstrate the retention or location of the virus ribonucleic acid. Giemsa has been used (5, 8, 9) as a specific stain to trace the location of ribonucleic acid in animal cells.

Two treatments designed to liberate the ribonucleic acid to the action of stains were: (a) Virus spots were treated with Lugol's[2] iodine and washed thoroughly in a 0.5 per cent solution of sodium thiosulphate, followed by distilled water. The treated spots gave a strong Millon's reaction.[3] (b) Virus spots were treated quickly with concentrated acetic acid instead of iodine, until they gave a weak Millon's reaction or none. If the treatment with acid was prolonged beyond a minute or two, the Millon's reaction was invariably negative. The negative Millon's reaction indicated that the protein fraction of virus had been split off and altered or leached away. The ribonucleic acid fraction, which is insoluble in concentrated acetic aid (6), was assumed to be left on the slide. When Giemsa stain was applied,

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whereas the untreated spots stained weakly, the iodine or acetic acid treated spots stained strongly (unless the dilution of Lugol's iodine had been considerably greater than 9:1). The color was blue, sometimes with a reddish or purple tinge. If the iodine was not washed out of the spot the stain was purple.

When iodine dissolved in absolute alcohol was applied and the spots were washed with 0.5 per cent sodium thiosulphate in 50 per cent alcohol solutions before staining with Giemsa, the spots were colored a somewhat lighter blue. If the iodine was not washed out, the color was pink to red. Generally the alcohol seemed to oppose the action of the iodine, presumably because it is a mild reducing agent and the iodine a mild oxidizing agent, or because of the solvent action of alcohol on iodine.

Thus, the staining reaction was similar after treatment in aqueous solutions of iodine-potassium iodide which only slightly altered the virus, and concentrated acetic acid, which presumably leached away the protein fraction (6). It is likely that the ribonucleic acid fraction of the virus took up much of the stain in the iodine-treated material.

Preliminary trials with fixed material. These were made mainly with pieces of tobacco leaves infected with tobacco mosaic virus, fixed in Karpechenko's fluid, embedded in paraffin, and sectioned at 10 or 12 μ. The sections were pretreated with Lugol's iodine or with a 2 per cent solution of iodine in absolute alcohol. They were then treated either with Giemsa's or Wright's stain. These two methylene-blue-eosin combinations gave similar results. Orange G in clove oil was generally used as a counter stain after differentiation in alcohol.

The main feature of these trials was that the striate material, derived by fixation from the crystalline inclusions of tobacco mosaic (3), stained a strong purple when pretreated with Lugol's iodine and washed with sodium thiosulphate. The color was like that obtained in spots of purified virus treated with iodine-potassium iodide, which had not afterwards been washed with sodium thiosulphate. Virus in the cell, or associated cell constituents, appeared to retain iodine more strongly than did similarly treated purified virus *in vitro*. Further, when the tissue sections were pretreated with iodine in absolute alcohol and washed with sodium thiosulphate in 50 per cent alcohol, the striate material stained pink, a color fainter but similar to that produced in spots of purified virus pretreated with iodine in alcohol and containing residual iodine.

In both instances amoeboid x-bodies stained much the same color as the chloroplasts, but more strongly. When the counter-stain was orange G, the chloroplasts, amoeboid x-bodies, and nucleoli were green, the chromatin of the nucleus was blue, and the cell walls faintly yellow. The purple color of the striate or crystalline material stood out very clearly.

In control sections of infected tissues not treated with iodine, the striate material had a trace of the yellow color from the orange G, but was only

faintly visible, and the x-bodies were rather lightly stained. In sections of
healthy leaves pretreated with iodine the colors of the normal cell constitu-
ents were similar to those of the same structures in diseased leaves, except
that sometimes the plastids stained somewhat less intensely than those in
diseased tissues.

A TECHNIQUE OF FIXING AND STAINING

Methods. After the preliminary trials, the material adopted for tests
of fixing and staining methods was epidermal strips, mainly from the leaves
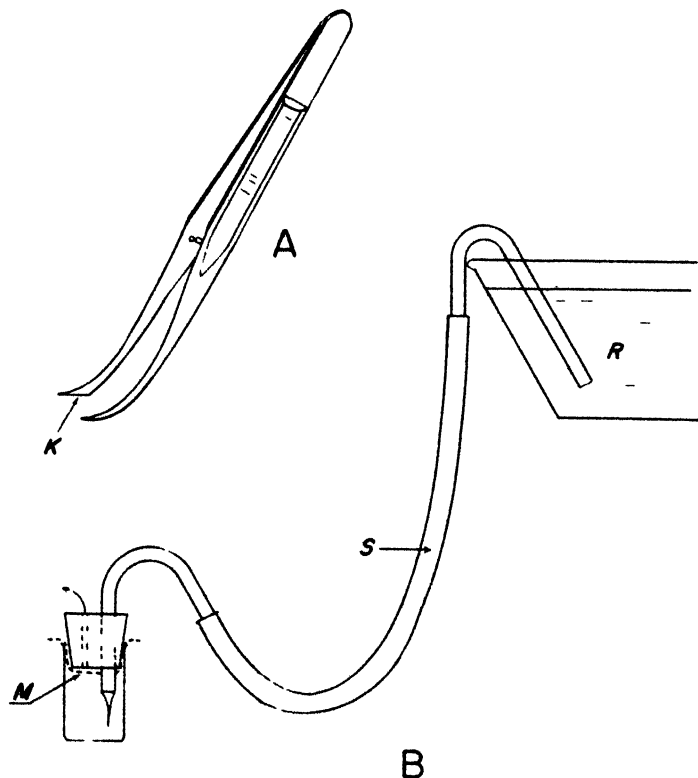
Fig. 1. A. Pair of stainless steel forceps prepared for making epidermal strips. The
curved edge of one arm is ground on the outside to a knife edge (K). B. Siphon arrange-
ment for washing epidermal strips and other material. Tap water is siphoned from
reservoir (R) through tube (S) into a vial containing the material to be washed. The
outlet from the vial is a hole in the stopper, covered by muslin (M).

of tobacco plants, healthy or infected with tobacco mosaic. Other hosts and
other viruses were also tested, and some sections made by hand or freezing
microtome were fixed and stained. Several lots of material were fixed and
embedded in paraffin. The convenience of the epidermal strip technique
and the ease and speed with which tests can be made were the reasons why
it was most often used in fixing and staining trials.

Following are some of the details of the technique finally adopted. The
epidermal strips were taken from the undersides of leaves, or from petioles

or stems. The instrument used was a pair of stainless steel forceps with smooth curved points. The lower outside edge of the arm further from the operator was ground to a knife edge and slightly turned so that it fitted smoothly against the curved end of the nearer arm (Fig. 1, A). The knife edge was pushed into the junction of two veins on the underside of a leaf or under the epidermis, the forceps closed, and a piece of the epidermis gently stripped off. Often very turgid leaves were easily stripped; if not, before stripping, they were allowed to wilt slightly, but not too much in case severe wilting affected the cell contents. When even wilted leaves could not be stripped, epidermis was sometimes stripped from petioles or stems. To prevent wound reactions in the epidermal cells, stripping was done under the fixative in a flat dish. Surgical rubber gloves were sometimes used to protect the fingers. Some trials were made stripping the epidermis under isotonic sugar solutions, but they were insufficient in number to determine whether this would prevent the secondary effects of wounding on the structure of intact cells. The epidermal strips were placed immediately in fresh fixative contained in small glass vials.

Washing with tap water when it was needed was done by siphoning from a flat dish into which water flowed directly from a tap (Fig. 1, B). The size of the siphon inlet and the level of the outflow from the vial determined the rate of flow, and were easily regulated. The delivery into the vial occurred a little more than half way down its length through a glass tube carried in a rubber stopper. The outlet hole through the stopper was covered by muslin (Fig. 1, B). To wash epidermal strips in a vial, water was started through one of the siphons, the end was plugged into the top of the vial, and the vial was placed in a test tube stand at a height that insured a desirable rate of flow.

A device used for staining paraffin sections on slides was the ring of a van Tiegham cell accurately ground to fit on the flat surface of the slide. The ground surface was dipped into glycerine, placed on the slide around the sections, and gently pressed to the surface. Stains dissolved in volatile liquids such as methyl alcohol can be held in this container for some time if it is covered with a cover slip. The ring can be lifted several times to allow a solution to run off, the sections can be washed, and the ring replaced and refilled with another solution.

c. Fixation and staining. The first fixative tried was simply Lugol's iodine diluted with an equal volume of absolute alcohol containing 2 per cent iodine. The fixative of this type adopted after a number of trials contained less iodine and some formalin, the formalin being added just before fixation was begun. The proportions were:

Lugol's iodine (6 gm. potassium iodide, 4 gm. iodine, 100 cc. water)	20 cc.
Water	25 cc.
Alcohol	50 cc.
Formalin (38–40 per cent formaldehyde)	5 cc.

This was designated fixative 1a.

The reasons for adopting this formula were: 50 per cent alcohol alone precipitates the virus; iodine alone, when it oxidizes the SH radicle of the virus, renders it insoluble (1); and the initial pH value of the mixture, about 3.6, is near the isoelectric point of purified virus and within the range where complete precipitation of the virus occurs in presence of cell components (2). This fixative appeared to maintain the virus inclusions of tobacco mosaic in much the same condition as in the living cells. Crystalline inclusions in recently infected tissues often were whole and without any sign of striations. Others were cracked, sometimes in mosaic patterns, but this may have been an effect of dehydration rather than of fixation. Virus which had apparently been diffused throughout the cytoplasm of the living cell often appeared to be fixed in disperse form, and could be stained the same color as the more concentrated inclusion bodies.

Formalin improved the fixation of cell contents, and a 1 in 20 concentration (2 per cent formaldehyde) was not high enough to interfere seriously with the oxidizing action of the iodine on the virus. Increasing the concentration to 1 in 10 without increasing the concentration of the iodine did interfere with the action of the iodine, and resulted in poorer staining of the virus. When the mixture was made up with 1 in 20 formalin neutralized by calcium carbonate, the pH value within a few minutes of preparation was around pH 5.2, and during a period of about 30 min. it fell to about 4.2. Presumably because the oxidation-reduction potential also was affected, inclusions in infected tissues fixed in this mixture failed to take up Giemsa stain, appearing like those in tissues fixed by standard methods.

The main disadvantage of this fixative for paraffin material is that, lacking the swelling component, acetic acid, it may be followed by shrinkage of the cells if it is not carefully handled, or the tissues may become brittle. Where even shrinkage of tissue components can occur, it is capable of producing useful results, for example, in leaves and leaf primordia; but where even shrinkage is not possible it may cause distortion of cell walls and cell contents. Also, the cytoplasm is flocculated, and the plastids may appear empty except for the starch grains.

This fixative may be followed by a Giemsa and orange G combination of stains, or iron alum haemotoxylin, trypan blue, etc. Only the Giemsa schedule is contained in this paper. Giemsa and orange G have given selective staining of virus in epidermal strips, but have been found somewhat more difficult to control when used on paraffin sections of growing tips.

The two stains may be applied together as a mixture. If they are, the orange G is prepared and kept separately as a 3 per cent solution in the same solvent as Giemsa, 3 parts methyl alcohol and 1 part glycerine. The stains are mixed immediately before application. They may be used undiluted or diluted 1 in 3 with methyl alcohol. The proportions have varied from 1 volume of orange G and 2 volumes of Giemsa to 1 volume of orange G and 19 of Giemsa. When the proportion of orange G is low, the staining

time may be lengthened and an intenser color obtained without too much displacement of the Giemsa dyes by the orange G.

The mixture of dyes, undiluted or diluted with methyl alcohol, is applied to the epidermal strips or sections in a vial for a period of from one to ten minutes. It is then diluted drop by drop with an equal volume of M/50 phosphate buffer of pH 7 (4, Table 41), and thereafter is left on the preparation for the same time or somewhat longer. The proportions and timing suitable for the material under examination may be found by a few preliminary trials.

In the resulting preparations of epidermal strips, the amoeboid bodies, as well as other virus inclusions, stain purple and the host nucleus stains greenish-blue to blue. Unlignified cell walls, for the most part, are barely outlined. The terminal cells of leaf hairs, even of healthy plants, may contain some purple material if the stains are not correctly balanced; but if they are, the contents of these cells, in the absence of virus, are generally green. The thick walls of guard cells may be blue-green or blue, and in sections, the walls of vessels are a light green. In good preparations of epidermal strips no purple-staining cell constituents have been found apart from those that can be interpreted as virus, except that sometimes the nucleoli are also purple. Nucleoli are said to contain ribonucleic acid (See (5) for a summary of information on this point). If the Giemsa is used alone, more intense staining is obtained, but other cell structures besides virus may also stain purple, or blue may be the predominant color throughout.

A staining schedule, that was applied successfully by students during a demonstration of these fixation and staining methods may be given as a working example. This schedule was not designed to preserve the leaf hairs, most of which collapse during the transfer from acetone to xylol, or xylol to balsam. The hairs may be preserved by increasing the period of fixation, hardening in 95 per cent alcohol, and using a graded series of acetone-xylol mixtures and an infiltration process for the change to balsam.

1.	Fix	30 min. or longer
2.	Wash in sodium thiosulphate solution, 0.25 per cent in 50 per cent alcohol	10 min.
3.	50 per cent alcohol, two changes	10 min.
4.	70 per cent alcohol	5 min.
5.	95 per cent alcohol	20 min.
6.	Giemsa stain, 4 parts, orange G, 1 part (0.2 to 0.5 ml.)	2 min.
7.	Dilute drop by drop with an equal quantity of M/50 phosphate buffer, pH 7	3 min.
8.	Rinse, Lugol's iodine 1 part in 99 parts of 80 per cent acetone	20 seconds or less
9.	Absolute acetone, three changes during	5 min.
10.	Xylol	5 min.
11.	Mount in neutral Canada balsam.	

The chief danger during differentiation (step 8) is of leaching out too much stain. Less rapid differentiation may be accomplished by passing the tissues rapidly through 70 per cent and 85 per cent acetone, or even directly to acetone. The more gradual the transition from stain to acetone the less the shrinkage of the epidermal strips; the more rapid, the stronger the staining. If the epidermal strips are washed thoroughly after fixation, and the stain is differentiated without iodine, virus may appear blue rather than purple. This may at times be considered desirable. A workable compromise for any particular purpose or lot of material can be discovered by trial.

SUMMARY

1. Spot tests with purified tobacco mosaic virus proved that pretreatment with iodine-potassium iodide solutions or concentrated acetic acid facilitates the staining of the virus with Giemsa stain.

2. Sections of tobacco leaves diseased with tobacco mosaic and fixed in Karpechenko's fluid were pretreated with iodine-potassium iodide solution and stained with Giemsa-orange G. The virus in the form of striate material stained purple.

3. A fixing and staining schedule was evolved on this basis that gave selective staining of tobacco mosaic virus inclusions and other more disperse material, presumably virus, in the cytoplasm. The test material was mainly epidermal strips from infected tobacco plants, and from normal controls.

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DEVELOPMENT OF THE LEAF SPOT FUNGUS IN THE OLIVE LEAF[1]

H. N. MILLER[2]

Boyer (2) in 1891 gave the first detailed description of the olive leaf spot fungus, *Cycloconium oleaginum* Cast. He noted its presence on both surfaces of the leaf, on the peduncle of the fruit, and very rarely on the fruit itself. He sectioned diseased leaves and described the growth of the fungus in the leaf cuticle. Brizi (3) in 1899 described the morphology of the fungus, illustrated the changes which it produced in the leaves of the olive, and investigated its life history extensively. Ducomet (5) in 1907 and Petri (7) in 1913 studied the development of the mycelium of *C. oleaginum* within the olive leaf and compared it with other intracuticular organisms.

Histological studies were made by Cavadas (4) in 1925, who reported that the mycelium penetrated all the tissues of the leaf by very fine prolongations.

Histological studies of the lesions in living leaves were made by the writer in an attempt to correlate growth of the fungus in the tissue with symptom development and conidial production. Other studies were concerned with the development of the fungus in fallen leaves.

MORPHOLOGY OF THE EPIDERMIS OF THE OLIVE LEAF

Petri (7) described the cuticle of the olive leaf as composed of five distinct layers: an outer, very thin wax stratum, the cuticle proper, two inside cuticular strata, and the layer of epidermal cells. He maintained that the fungus *Cycloconium oleaginum*, growing between the two cuticular layers, secretes enzymes capable of dissolving the cutin, which it utilizes as a source of energy.

To determine the morphological structure of the epidermis, microchemical and optical tests of sections of healthy olive leaves were made by the methods described by Rawlins (8). The leaf material was killed in formalin-acetic-acid-alcohol mixture, embedded in paraffin, sectioned 12 to 14 μ thick, mounted on slides, and treated with xylene and alcohol.

A cross section of a healthy mature leaf, treated with zinc-chlor-iodide or iodine-potassium iodide followed by 65 per cent sulfuric acid, revealed four differently stained layers within the cuticular and epidermal region. The outer, very thin dark wax layer and beneath it the cuticle proper, which

together averaged 3 μ thick, stained yellow. Under the cuticle was a layer which stained dark orange to brown, and below this the innermost layer, composed of epidermal cells which stained blue in the regions of the cell walls.

Cross sections of healthy leaves were mounted on slides in water and studied with polarized light. When the section was rotated at an angle of 45° with the vibration axis of the analyzer, three anisotropic tangential layers were visible within the epidermal region (Fig. 1, A). Rotation of the field to the 90° angle position showed anisotropic radial layers extending
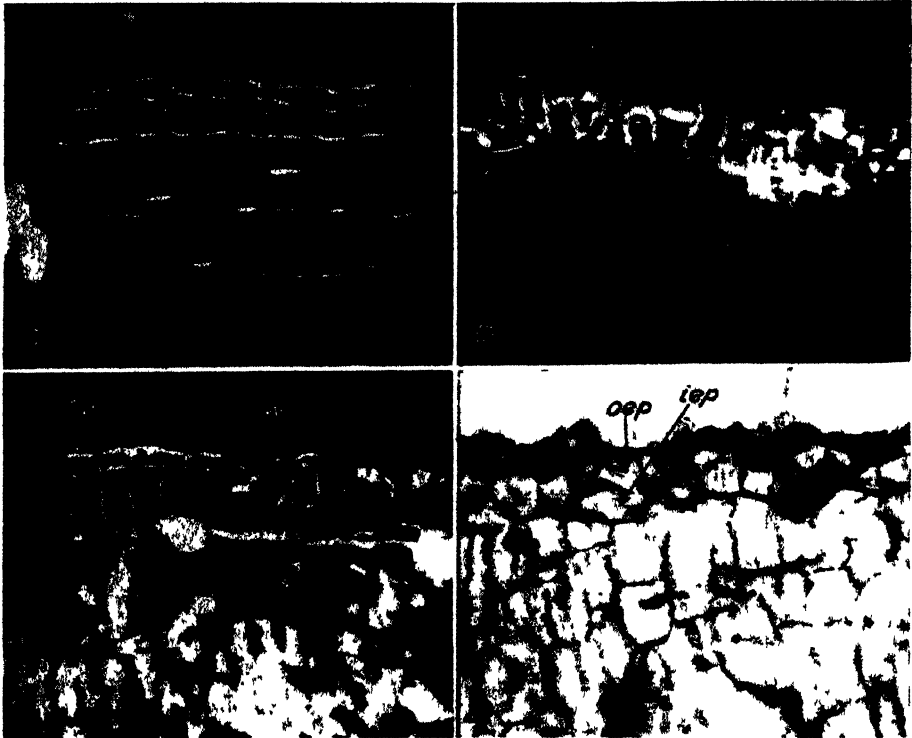
FIG. 1. Cross sections through the epidermis of a normal mature olive leaf (all ×270). A. Photographed under crossed Nicol prisms with the section rotated at an angle of 45° with the vibration axis of the analyzer to show the tangential walls (tw) of the two epidermal cell layers. B. Photographed with field rotated to the 90° position to show radial walls (rw) of the epidermal cells. C. Taken at a plane of rotation to show some tangential and radial walls of the two layers of epidermal cells. D. Section treated with KOH to remove cutin and waxes, then stained with Delafield's hematoxylin to show the two layers, (oep) outer epidermal cells and (iep) inner epidermal cells.

from the lower edge of the epidermis to the layer of cuticle (Fig. 1, B). These doubly refractive layers were regarded as the tangential and radial walls of two layers of epidermal cells. The anisotropy of the layers probably results from the presence of cellulose, because cellulose is doubly refractive or anisotropic when viewed between crossed Nicol prisms.

The photograph in figure 1, C, was taken at a plane of rotation between crossed Nicols in which some radial and some tangential walls of the epi-

dermal layers were anisotropic. These observations indicate that the layer which lies just beneath the cuticle proper, and which stains dark orange or brown with zinc-chlor-iodide, is cellular in nature and constitutes a second layer of epidermal cells above the layer easily recognized as epidermis. Failure of the walls in this cell layer to stain blue with a cellulose stain is due to their impregnation with cutin. The cutin probably accounts for the fact that the cellular nature of this epidermal layer is not clearly apparent upon examination with the ordinary microscope.

Attempts were made to remove the cutin and other fatty materials from the epidermis of the leaf sections. The cutin was insoluble in ether and benzene. After the sections were submersed in ether for 24 hr. some slight clearing occurred within the outer layer of epidermal cells. In sections auto-claved in alcoholic KOH at 15 lb. pressure for one hour, varying degrees of clearing occurred, and in thin sections almost complete hydrolysis of the cutin and fatty materials had taken place. The cleared sections were washed in water for 30 min. and then stained with Delafield's hematoxylin. The walls of the two layers of epidermal cells remained intact although somewhat distorted by the treatment (Fig. 1, D).

These studies indicate that the epidermis of a mature olive leaf is com-posed of an outer thin wax layer, a layer of cutin (hereafter called the cuticle proper), and two layers of epidermal cells, the outer impregnated with cutin and fatty materials. The cells of the two layers are not similar, those of the innermost layer being elongated radially. The cell walls in the two layers may or may not occupy parallel planes.

INITIATION OF INFECTION AND GROWTH OF THE FUNGUS IN LIVING LEAVES

Information on the time of year when natural infection occurs and symptoms develop was inadequate. Petri (7) maintained that the fungus develops mainly at the end of summer and the beginning of spring. Hayne (6), who first reported on the disease in California, stated that the fungus appears on the leaves in any season of the year, and that lesions in all stages of development sometimes are found on the same leaf. He stated further, however, that the disease usually makes its appearance at the end of summer or in autumn. In the winter of 1946–47, Wilson and Miller (9) studied in-fection periods in central California by exposing small potted olive trees in an orchard under leaf-spot-infected trees for a time and then removing them to a lath house. With favorable moisture and temperature, infection was initiated at various times between late November and late February, but in none of these particular tests did lesions become visible until early spring. Judging from these data, conidia of *Cycloconium oleaginum* germinate at temperatures between 9° and 25° C., with the optimum near 16° C.; and the mycelium grows at temperatures between 12° and 30° C.

To study the growth of the fungus in living leaves, infected material was collected at regular intervals over a twelve-month period. Pieces cut from the lesions were killed in formalin-acetic-acid-alcohol mixture and embedded in paraffin.

For very young lesions, before the hyphae of the fungus had assumed the dark brown color typical of older mycelium, cotton blue was the most satisfactory stain. After staining, the sections were differentiated with 95 per cent alcohol and cleared in clove oil. Where greater differentiation between the fungus and the cellular structure of the leaf was desired, a combination of safranin and fast green or of bismark brown and methyl violet was used. Staining with Sudan IV revealed the path of the fungus hyphae through the cuticle. The mycelium and spores in old lesions, were dark brown to brick red in advanced stages of development and could be seen and studied easily without the use of stains.

It has been assumed that germ tubes of *Cycloconium oleaginum* conidia pass through the leaf cuticle, but the writer was unable to find a germinated conidium on the leaf surface with germ tube penetrating the cuticle. Petri (6) maintained that the hyphae of *C. oleaginum* secrete enzymes which dissolve the cuticle in the immediate region of penetration.

FIG. 2. Cross sections of Cycloconium infected olive leaf (both × 270). A. Hyphae (hy) within the cells of the outer epidermal layer. B. Mycelium (m) within the cells of the outer epidermal layer and conidia (c) on the leaf surface. The cellular nature of the epidermal layer which the fungus occupies is not evident from the picture, because the outer layer of the epidermal cells is impregnated with cutin or fatty materials which prohibit absorption of the stain used.

In an area where infection had occurred recently, hyphae were abundant in the outer layer of epidermal cells. These hyphae had developed tangentially within the epidermal cells of the upper layer and were both intracellular and intercellular (Fig. 2). They were light brown, uniform in diameter, and continuous for considerable distances. The mycelium layer in the outer epidermal cells was one hypha thick, and followed closely the surface contour of the epidermal cells except where numerous hyphal branches arose and penetrated the cuticle to the outside. These external hyphae bore conidia directly or expanded into bulbous conidiophores on which the conidia were borne. At this stage of development the mycelium in the leaf had not penetrated below the first layer of epidermal cells. No breakdown in or injury to the cells of the leaf was apparent. It is assumed

that the nutrients required for fungus growth are obtainable in this area of the epidermis.

Lesions, when first visible, are very small, brown sooty blotches on the leaf surface. Leaf pieces with lesions in this stage of development were cleared by boiling in 70 per cent alcohol to remove the chlorophyll and then were boiled in 5 per cent sodium hydroxide. Microscopical examination of the cleared leaf pieces showed the presence of some mycelium on the surface. The scattered hyphae were composed of short, thick, dark brown cells. The sooty appearance of the lesions, however, was due in large measure to the numerous conidia.

Leaf lesions which appeared in early spring continued to enlarge and develop during April and May, and often coalesced to cover a considerable area of the leaf surface. Color of the lesions did not change during this period, because the sooty brown to black spores were abundant.   •

During the summer the mycelium became darker brown and more septate. The cuticle and outer walls of the epidermal cells of the leaf became thicker, as did the mycelial layer within the outer layer of epidermal cells; and prolongations of the hyphae extended between the radial walls of the inner layer of epidermal cells. No light-colored hyphae, characteristic of actively growing fungus, were found. Such a condition is consistent with the disease picture, inasmuch as growth and expansion of the leaf lesions apparently cease during the hot, dry summer months.

Severely infected leaves fall during late spring and early summer. On leaves remaining on the tree during the summer, the yellowed leaf area surrounding a lesion was a striking contrast to the brown lesion with its series of more or less concentric zones. Slowly developing lesions remained as gray-to-black spots on a green leaf and failed to produce conidia.

In summer, moreover, the lesions became dry, hardened, cracked or blistered, and scarlike. The cuticle broke away from the epidermal cells and in the center of the spot the outer epidermal cell walls sloughed off. This effect was apparently produced by the fungus since the mycelium had grown throughout the outer epidermal cells. Other hyphae had grown between cells of the inner epidermal layer and along the end walls of the palisade cells, in many cases isolating single cells. Only rarely in this stage of development did the fungus grow into the lumen of the cells of the inner epidermal layer or into the palisade cells. Some of the epidermal cells were filled with a light brown granular material which failed to take the stains used.

In autumn, some of the lesions on leaves that had remained on the trees expanded at their margins. These margins of newly involved tissue were brown and bore abundant conidia. New mycelium that had extended from the periphery of old lesions into adjacent tissue had much the appearance of mycelium found in early stages of lesion development. The new hyphae extended singly and tangentially along the outer layer of epidermal cells, and numerous sporophores arose from these hyphae, passed through the

cuticle to the outside, and bore conidia at the surface of the leaf. These expanded lesions were an important source of inoculum for new infections in late fall (9). However, such an expansion of old leaf spots did not always occur.

Many small lesions over which the cuticle of the leaf had not disappeared during the summer again became active during autumn and produced spores over the entire surface of the lesions; these likewise provided a supply of spores for new infections. Such lesions apparently were initiated in late spring, and before they had developed to any great extent their growth was arrested, probably by high temperatures.

So far as was known there was no other effective source of inoculum (9). The old lesions which resumed growth in the fall of the year probably provided an adequate supply of conidia to account for subsequent infection. However, some workers believed that fallen leaves on the ground might be
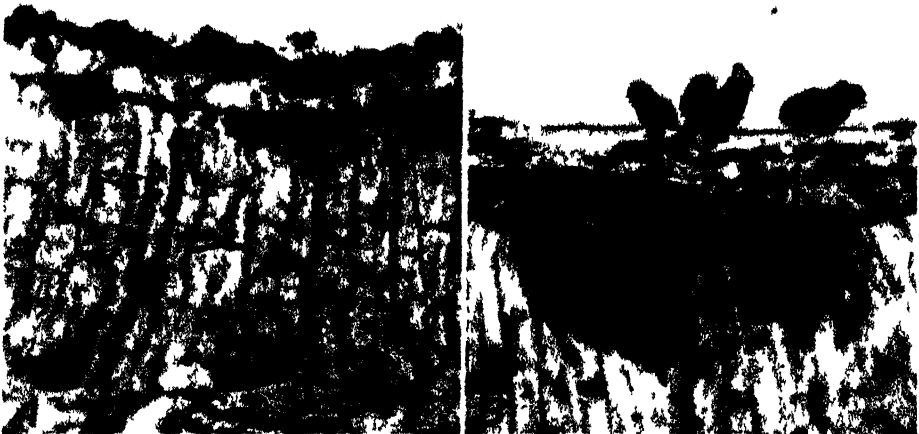
FIG. 3. Cross sections of dead olive leaf invaded by Cycloconium (both ×270). A. Mycelium growing throughout the epidermal cells and hyphae extending down between the palisade cells B. Stromatic mass of fungus cells growing in the tissue of the leaf. Typical conidia of *Cycloconium oleaginum* on hyphae growing out of the fungus tissue.

a source of inoculum. Bernes (1), for example, recommended burning the leaves as a control measure.

GROWTH OF THE FUNGUS IN DEAD LEAVES

To study the behavior of the fungus in fallen dead leaves, affected leaves were collected from the ground in the spring of the year and at various times throughout the late fall and early winter. Some of the leaves had been on the ground for several months while others had fallen recently. Judging from microscopical examination of sections of such material, the fungus did not die when the leaves fell, but remained alive for some time growing as a saprophyte. The behavior of the fungus in dead leaves differed markedly from that in living leaves in that the mycelium ramified throughout the leaf tissue even to the lower epidermis (Fig. 3, A). In the leaves that had not

been on the ground long enough to disintegrate and to be overrun by other fungi, conidia of *Cycloconium oleaginum* were produced in abundance. The time of this conidial production corresponded with that on leaves in the trees.

Although examination of numerous sections failed to show a sexual stage of the fungus, certain stromatic bodies were found (Fig. 3, B). The significance of these in the life history of the organism was not ascertained. They were composed of a basal portion of pseudo-parenchymatous mycelium which was embedded deeply in the palisade tissue of the leaf, and a top portion in the subcuticular region of the leaf composed of closely entwined hyphae. In many cases, sporophores arose from this mycelial layer, passed through the cuticle, and produced conidia typical of *Cycloconium oleaginum*. In other instances the stromatic mass had greatly expanded and broken through the leaf tissues to the outer surface. Since other fungi grew abundantly on the dead olive leaves, it is difficult to state definitely that the stromatic bodies were structures of *C. oleaginum*. Such bodies were found in leaf lesions which appeared to be free of any fungi, and hyphae and conidia typical of *C. oleaginum* were closely associated with them. They developed during the saprophytic life of the fungus or when the fungus was subjected to adverse conditions. No similar structures have been found associated with the fungus on living leaves. Furthermore, Wilson and Miller (9) found similar stromatic tissue in pure cultures of *C. oleaginum*.

DISCUSSION

Cycloconium oleaginum has hitherto been considered an intracuticular organism which by the secretion of enzymes is able to utilize the cutin as a source of energy. The results of the present investigation demonstrated that the epidermis of an olive leaf is composed of two layers of epidermal cells and that the fungus grows in the cells of the outer layer. Consequently, the fungus probably utilizes materials within such cells as food sources.

Initial infection and development of symptoms of the olive leaf spot can be correlated with growth of the fungus in the leaf tissue. In late fall, winter, and early spring when conditions favor infection, the fungus is expanding in the leaf and producing abundant conidia. The fungus is more or less inactive during the summer. It survives in many of the leaves which remain in the tree, resumes its growth in late fall, and again produces conidia.

In dead leaves on the ground, *Cycloconium oleaginum* may remain alive for some time, undergoing extensive saprophytic development. Conidia may be produced in abundance on these leaves. It is doubtful, however, if these spores are an important source of inoculum. Judging from field observations (9), conidia of *C. oleaginum* are carried short distances by the wind or are disseminated in water droplets which are spattered around. In a dry state the conidia are not easily detached from the conidiophores by air currents and, for this reason, perhaps are not widely disseminated by wind.

SUMMARY

The epidermis of a mature olive leaf is composed of an outer thin wax layer, a layer of cutin, and two layers of epidermal cells. The upper layer of epidermal cells is embedded in cutin and other fatty materials which can be removed by autoclaving in alcoholic KOH. The walls of this layer of cells possess the same refractive index as the cutin, fail to take the cellulose stains, and are not visible in sections until the cutin is removed. The cell walls are visible, however, when viewed in polarized light.

The mycelium of *Cycloconium oleaginum* develops one-hypha-thick in the outermost layer of epidermal cells and is both intercellular and intracellular. Short hyphal branches arise along the mycelium, grow through the cuticle to the leaf surface, and produce single spores of one or two cells.

Growth of the fungus in the leaves during the early summer months proceeds at a slow rate. The mycelium ramifies through the leaf tissue and between the cells of the inner epidermal layer. No conidia are produced during the summer.

In the fall of the year, some of these inactive lesions expand and produce spores on their margins. Other lesions of more recent origin do not expand, but become active and produce spores over their entire surface.

The fungus remains alive and continues to grow as a saprophyte on olive leaves after they have fallen to the ground. Here it develops extensively in the host tissue and, for a time after the leaf falls, produces conidia.

No perfect stage. was found in these studies, but certain stromatic bodies associated with the fungus were found in dead olive leaves bearing old lesions.

Apparently infection and development of lesions are influenced by moisture and temperature and, in California, may occur at any time during fall, winter, or early spring when such conditions are favorable.

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PHYTOPATHOLOGICAL NOTES

Parasitism of Citrus in Florida by Various Species of Dodder, Including Cuscuta Boldinghii Urb., a Species Newly Reported for the United States. Cuscutas on citrus have been reported in the past, but in the United States only George[1], Stout[2], and Bellue[3] have listed specific names of the dodders involved. George's report mentions the attack of citrus in Arizona by *Cuscuta gronovii latiflora,* and Stout's and Bellue's reports mention the parasitism in California of *Citrus sinensis* (L.) Osbeck by *Cuscuta subinclusa.* An emendation is suggested by Dr. Freeman Weiss[4] to the reference in his Check List Revision to *Cuscuta americana* L. as a parasite of *Citrus* spp. in California and Florida. According to Dr. Weiss, the record of this dodder, originally made by Nowell in Diseases of Crop Plants in the Lesser Antilles, should have been restricted to the West Indies where this species is endemic. Lacking reports of an identified species on citrus, the record for the states above mentioned, including Arizona, should have read *Cuscuta* sp. indet.

Abroad, specific determinations of dodder affecting citrus are limited to the reports of Gandara[5] in Mexico and Nowell[6] in the Lesser Antilles, who mention orange and lime respectively as being parasitized by *Cuscuta americana.*

In Florida the writer has observed three species of dodder on citrus and a near-citrus species: (1) *Cuscuta americana* L. on *Citrus sinensis* (L.) Osbeck (sweet orange), *Citrus paradisi* Macf. (grapefruit), *Citrus Limon* (L.) Burm. (lemon), and *Citrus aurantifolia* (Christm.) Swingle (Tahiti lime); (2) *Cuscuta campestris* Yunck. on *Fortunella margarita* (Lour.) Swingle (Nagami kumquat); and (3) *Cuscuta Boldinghii* Urb. on *Citrus sinensis.*

The above three dodders were identified through the courtesy of Dr. T. G. Yuncker, De Pauw University. Concerning *Cuscuta Boldinghii,* he writes as of September 29, 1948: "Your other specimen collected at Tavares is most interesting as it is the first report of this species in the U. S. A. It is *C. Boldinghii* Urb. The type was from Bonaire. I also have it from Curacao, Yucatan, Mexico, Haiti and Honduras . . . [as well as] from Guatemala and Costa Rica. . . . None of my former records report it on Citrus."

Regarding the incidence of these three species of dodder on Florida citrus, *Cuscuta Boldinghii* has been found at only one location, in Lake

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[1] George, D. C. A parasitic dodder on citrus and olive. Arizona Comm. Agr. and Hort. Ann. Rept. 9: 65–66. 1917.

[2] Stout, G. L. A case of dodder (*Cuscuta subinclusa* Durand and Hilgard) on Valencia orange (*Citrus sinensis* Osbeck) in Southern California. California Dept. Agr. Bul. 29: 121–124. 1940.

[3] Bellue, M. K. Record of dodder (*Cuscuta subinclusa* Durand and Hilgard) on citrus in Orange County. California Dept. Agr. Bul. 29: 145. 1940.

[4] Weiss, Freeman. Check List Revision, U. S. Dept. Agr., Pl. Dis. Reptr. 25: 36–46. 1941. A letter from Dr. Weiss dated Oct. 11, 1948, gives the above correction.

[5] Gandara, G. Enfermedades y plagas del naranjo. Estac. Agr. Centr. Mexico Bol. 111: 5–40. 1920.

[6] Nowell, W. Diseases of crop-plants in the Lesser Antilles. 383 pp. The West India Comm. London. 1923.

411

County, where it was growing mainly on the cover crop and only weakly on an overhanging branch of orange.

Cuscuta campestris, similarly, has been observed but once, in Polk County, growing but sparingly on Nagami kumquat.

Cuscuta americana, in contrast to the two foregoing species, is generally distributed, grows vigorously on citrus, and once established covers a tree with a matting so heavy and tenacious (Fig. 1) that its eradication becomes a matter of considerable cost. According to records of one fruit-producing company, the hand removal of dodder from a single tree last year entailed an expenditure of 48 man hours.—L. CARL KNORR, Citrus Experiment Station, University of Florida, Lake Alfred, Florida.

FIG. 1. Parasitism of *Citrus sinensis* by *Cuscuta americana*.

A Simple Power-Operated Atomizer for Applying Small Quantities of Concentrated Spray Fluids.—In the course of experimental work on tar acid compounds for end of season killing of potato vines[1], it became necessary to apply small quantities (10–50 ml.) of many different concentrated liquids to plots of six plants each. Hand-operated atomizers proved both slow and tiring to the operator and the necessity for repeated washing out of the container was another disadvantage. As the measured quantities of the sprays were carried to the field in $6 \times \frac{3}{4}$-in. boiling tubes it was decided to make a small atomizer of the scent spray type, using these tubes

[1] Wilson, A. R., A. E. W. Boyd, J. G. Mitchell, and W. S. Greaves. Potato haulm destruction with special reference to the use of tar acid compounds. Ann. Appl. Biol. 34: 1–33. 1947.

as interchangeable containers. As an automobile was usually available on the experimental field, a convenient source of pressure was obtained by leading the exhaust gases to the plots by means of a length of hose pipe. This system has proved so satisfactory that it seems likely to be of use to anyone engaged on similar work where contamination with exhaust gases and small quantities of condensation water is immaterial.

Two lengths of 1-in.-wide, light gauge, tinned iron are bent as shown in figure 1 to form a spring cradle for the container. The two halves are riveted together at (a) so that the bottom half (c) hinges on the top half (b). When the cradle is closed, the base of the container (d) is held in the
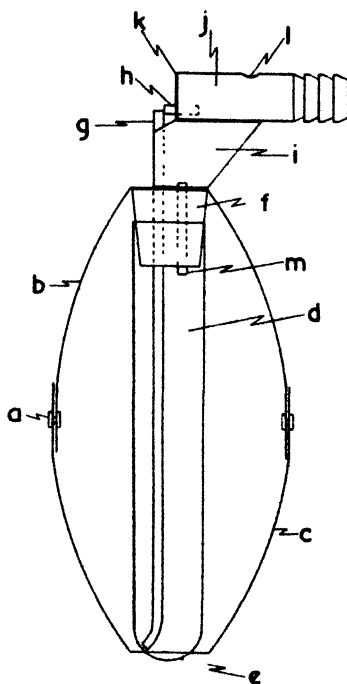
FIG. 1. Side elevation of atomizer (to scale).

depression (e) while the top is pressed firmly on to the rubber bung (f). The feed (g) and pressure (h) pipes are of $\frac{1}{8}$-in.-bore copper tubing firmly held at the correct angle to each other by two pieces of tinned iron (i) soldered, either side of the feed pipe, to the top of the cradle and to the underside of the hose connection link (j), respectively. The end of the hose connection link is blocked by a disk of tinned iron (k) through which is soldered the pressure pipe. A $\frac{1}{4}$-in. hole is drilled at (l) to serve as a pressure release valve. A short length of $\frac{1}{8}$-in.-bore copper tubing (m) serves the dual purpose of preventing the development of negative pressure in the container and stopping rotation of the bung on the feed pipe.

To operate the atomizer, the hose line from the exhaust is pressed on to the hose connection link and the thumb is held over the hole (l). Less than 0.5 ml. of spray fluid remains in each container after use.

Since the work described was completed, details of another apparatus designed for a similar purpose, but not on the atomizer principle, have been published.[2]—A. R. WILSON, Agricultural Research Council, Potato Storage Investigation, care of Nottingham University, School of Agriculture, Sutton Bonington, Loughborough, England, and A. E. W. BOYD, now at The Department of Agriculture for Scotland, Plant Pathology Laboratory, East Craigs, Corstorphine, Edinburgh.

A Method for Preserving and Mounting Pressed Leaves.[1] There has been a long-felt need for a simple and convenient method of preserving and mounting leaves without loss of color and detail. Also it is frequently desirable to be able to see both surfaces of a leaf or to view it with transmitted light, particularly if one wishes to study symptoms of virus or other diseases. Herbarium specimens, either in conventional mounts or unmounted, are not entirely satisfactory for class purposes, since dried leaves are too brittle for frequent handling and their distinctive markings are likely to fade or to disappear.

After considerable experimentation, the following method of mounting has been devised to overcome some of the aforementioned disadvantages. Leaves showing the desired characteristics are pressed and dried in the usual way as soon as possible after collection. At this stage, the use of heavy, corrugated cardboard interleaves has proven advantageous. The corrugations should run in the same direction in all interleaves, to facilitate air circulation through the press. The pressure exerted on the press should be evenly distributed and sufficient to permit the specimens to dry without wrinkling.

When the leaves are thoroughly dried, a coat of medium thin white shellac is applied with a camel's-hair brush to both surfaces. The shellacked leaves can be placed to dry overnight on a sheet of waxed paper or on a coarse wire mesh. The shellac has the effect of restoring the lustre and color of the leaf and of giving markings and patterns a remarkably natural appearance. The specimens are then arranged on the adhesive side of a sheet of glossy Permafilm[2] or similar preparation, and covered with a duplicate sheet applied according to the manufacturer's directions. The application of the covering sheet requires some patience and care in order to avoid wrinkling and air spaces where the films come in contact with each other. A mistake at this stage is irremediable. If air is entrapped between the leaf and the transparent sheet it can be removed by making a needle puncture at the edge of the leaf or at the end of the petiole and exerting pressure on the mount either by rubbing with a soft cloth or by means of a photographic

[2] Davis, J. F. A sprayer attachment for rapidly applying small quantities of spray materials. Jour. Amer. Soc. Agron. 39: 835–836. 1947.

[1] Contribution No. 966 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] Permafilm is the trade name used by Denoyer-Geppert Co., Chicago, Ill. It is probable that other films of a similar type are also obtainable.

print roller. The resulting transparent mount can then be trimmed to size. Leaves in this mount are reasonably flexible and can be handled safely without danger of breakage. The transparent portions may be silghtly cloudy at first but the cloudiness disappears in the course of a few days.

Mounts measuring, when trimmed, 4–7/8 × 7–3/4 in. can be made satisfactorily and are more easily filed than larger sizes. They can be attached to 5 × 8-in. filing cards. This can be done by laying on a table the card and the mount face up and end to end, separated by a 1/8-in. gap. Juncture is made with 1-in.-wide strips of Permafilm applied both back and front. The mount can then be folded back on the card, ready for filing. A description of the specimen or other notes can be typed on the face of the card before attaching the mount.—R. S. WILLISON, Box 28, St. Catharines, Ontario, Canada.

BEET-MOSAIC VIRUS–GREEN PEACH APHID RELATIONSHIPS

Edward S. Sylvester [1]

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The relationships between the non-persistent (6) viruses and their vectors have been the object of research of various authors (1, 2, 4). In several instances these relationships are well defined; e.g., the influence of starvation upon vector efficiency, and the lack of prolonged retention. To date no critical data have been gathered upon such points as the acquisition and inoculation thresholds (3), the effect of short pre- and post-infection feeding starvation periods, and the influence of the number and duration of stylet insertions, or punctures, upon transmission. Data upon such points are presented in this paper.

MATERIALS AND METHODS

In the following experiments with the beet-mosaic virus, a total of 6 replications, 5 plants per treatment, for each variation were made. Single previously noninfective green peach apterae served as the vector, and they were transferred by the camel's hair brush technique. Where possible, a single virus source was used in each series of replications. The test plants were seedling beets in the cotyledon stage. In all cases where time was taken in seconds, a stop watch was used.

ACQUISITION THRESHOLD

The acquisition threshold refers to the minimum determinable time required by a feeding vector to acquire virus from a source plant. To determine the acquisition threshold of the beet-mosaic in sugar beets, using the green peach aphid as the vector, a series of 5-sec. interval infection-feedings was used. The first interval was a 5-sec. and the last a 30-sec. period. The data obtained in two separate tests are given in table 1.

The results of the two tests indicate that the acquisition threshold of this particular vector-virus association is between 6 and 10 sec. There appears to be an improvement in efficiency if the penetration is maintained for more than 10 sec., but not beyond 15 sec. Since test I suggested the possibility of either an exceptionally high value for the 15-sec. interval, or an exceptionally low value for the 20-sec. interval, a second test was made. Using the mean value from the two separate tests, it was concluded that feeding punctures maintained beyond the 15-sec. interval are of little value in improving efficiency.

Other results have indicated that a 5-min. infection feeding has no advantage over a 30-sec. infection feeding. Consequently, it appears that the first 15 sec. is the crucial period, as far as the length of infection feeding

[1] Junior Entomologist in the Experiment Station and Instructor in Entomology, University of California, Berkeley, California.

TABLE 1.—*Results* of trials to determine the acquisition threshold of the beet-mosaic virus, using starved single green peach aphid apterae, a variable infection feeding, and a 0.5- to 2-hr. test feeding period. In each test, a total of 6 replications, 5 plants per treatment, was used

Test	Infection feeding period in seconds						Total
	5	10	15	20	25	30	
I	0/30	7/30	19/30	13/30	20/30	21/30	80/180
II	0/30	4/30	11/30	12/30	5/30	7/30	39/180
Mean	0/30	5.5/30	15/30	12.5/30	12.5/30	14/30	59.5/180

* In the ratios listed, numerator is number of plants infected; denominator, number of plants inoculated.

is concerned. This feeding period is only one of many factors which will influence the vector efficiency in any given test.

INOCULATION THRESHOLD

The trials for determining the inoculation threshold were done in the same manner as in the case of those of the acquisition threshold, with the exception that the infection feeding remained relatively constant, while the test feeding was varied. As in the acquisition threshold tests, the intervals used were 5 sec. apart, commencing with a 5-sec. test feeding and terminating with a 30-sec. period. The results of the trials are given in table 2.

The results indicate that the inoculation threshold lies between 6 and 10 sec. One difference in these data from those taken on the acquisition threshold was the lower level of transmission obtained in the inoculation threshold trials. Also there was little change in efficiency of transmission until the puncture of the test feeding period had been established for more than 25 seconds.

The lower level of transmission in the inoculation threshold trials apparently was a direct result of the activity of the vector on the test plant, since in the inoculation threshold test and the acquisition threshold test I replications, the virus source was common, as was the aphid colony. The test plants were taken from the same lot, and the comparative replications were done on the same day.

TABLE 2.—*Results* of trials to determine the inoculation threshold of the beet-mosaic virus, using starved single green peach aphid apterae, a single virus source, a 0.5- to 3-min. infection feeding period, and a variable test feeding period

	Test feeding period in seconds						Total
	5	10	15	20	25	30	
Result	0/30	7/30	10/30	8/30	8/30	15/30	48/180

* In the ratios listed, numerator is number of plants infected; denominator, number of plants inoculated.

TRANSMISSION THRESHOLD

The term transmission threshold, which heretofore has not been defined, refers in this work to the minimum determinable period of time required for a single vector to transmit a virus from a disease source to a healthy plant. In the experiments to determine a transmission threshold value the following three variations were used: (1) an infection and test feeding period each 10 sec. in duration, (2) an infection and test feeding period each 15 sec. in duration, and (3) an infection feeding period of 15 sec. and a test feeding period of 20 sec. Again in this series of tests, 6 replications, 5 plants per treatment, for each of the 3 variations, were used. Only the data from the positive results are included in table 3.

TABLE 3.—*Results of test to determine the transmission threshold of the beet-mosaic virus using starved single green peach aphid apterae. The pre-infection feeding starvation period varied from 1.5 to 3.5 hr.; the infection feeding and test feeding intervals varied with the experiment. Only the data from the positive results are included*

IF: TF[a] (in seconds)			Results[b]	Transfer time[c]		Total time (in seconds)	
Intended	Actual	Mean		Range	Mean	Range	Mean
10: 10	10: 10	10: 10	4/30	23–87	42.25	43–107	62.25
15: 15	15: 10–15	15: 14.2	9/30	19–54	25.8	42–84	55.0
15: 20	15: 9–15	15: 16.8	10/30	15–37	23.8	45–67	55.6

[a] The IF: TF ratio expresses the second intervals that were used for the infection feeding and the test feeding periods, respectively.
[b] In the ratios listed under results, numerator is number of plants infected; denominator, number of plants inoculated.
[c] The term transfer time refers to the time elapsing between the termination of the infection feeding period on the virus source and the beginning of the test feeding period on the healthy plant.

The results obtained give a minimum value for transmission, or a transmission threshold, of 42 sec. The maximum in these trials was 107 sec., with a transmission threshold mean of 56.5 sec. Obviously the maximum value obtainable would vary considerably, depending upon the non-feeding activities of the individuals being used. The minimum value obtainable would be limited by at least three factors: (1) the time needed for attaining the acquisition and inoculation thresholds, (2) the time required by the manual act of transferring, and (3) the time consumed in an orientation period, apparently required by the insects after being transferred.

INFLUENCE OF THE LENGTH OF THE PRE-INFECTION
FEEDING STARVATION PERIOD

In the initial experiment upon this phenomenon, the aphids were starved in a vial prior to the infection feeding for the following time intervals: 0, ¼, ½, 1, 2, 3, 4, 5, and 6 hr. The infection feeding following starvation was approximately 30 sec., and the test feeding period was approximately 1 hr. in duration. The results of the trials are given in table 4.

TABLE 4.—*Results* of *trials to determine the influence of the length of the pre-infection feeding starvation period upon vector efficiency. Single green peach aphid apterae fed, after the starvation period, upon a single virus source were used. The infection feeding was approximately 30 sec.; the test feeding approximately 1 hr.*

	Starvation time in hours									Total
	0	¼	½	1	2	3	4	5	6	
Result	3/30[b]	22/30	20/30	20/30	18/30	24/30	21/30	21/30	26/30	178/300

[a] In the ratios listed, numerator is number of plants infected; denominator, number of plants inoculated.
[b] The result is the mean of 2 replicated check series

From the table it appears that the maximum effect of pre-infection feeding starvation can be obtained in a period of 15 min., and that little benefit is to be gained by increasing the length of this period to a matter of hours. Watson (5), using intervals of 0, 1, and 20 hr., obtained similar results.

Since the maximum effect obtained in the experiment was found in 15 min., a replicated series was done to test the intervals of time occurring between 0 and 15 min. The intervals used were: 0, 2, 5, 10, 15, and 60 min. The results are found in table 5.

In this case, the maximum effect of starvation was found to occur after 5 min. starvation, and but little, if any, increase was obtained for a 2-min. period. It appears that the effect of starvation upon the transmission efficiency occurs rather rapidly and suddenly, and if the action of any inhibitor or inactivator is responsible for the phenomenon, as has been suggested by Watson and Roberts (6), it is potent and rapid in its action.

INFLUENCE OF POST-INFECTION FEEDING STARVATION ON TRANSMISSION

Watson (5) reported that the beet-mosaic virus was lost most rapidly in the first few hours of post-infection feeding starvation, but that the transmission of beet-mosaic virus was less influenced by this treatment than were some of the other non-persistent viruses. Her evidence indicated that 22 per cent of the initially infective aphids were still infective after 20 hr. fasting.

TABLE 5.—*Results* of *trials to determine the influence of the length of the pre-infection feeding starvation period upon vector efficiency. Single green peach aphid apterae, fed, after the starvation period, upon a single virus source were used. The infection feeding was approximately 30 sec.; the test feeding 1 hr.*

	Starvation time in minutes						Total
	0	2	5	10	15	60	
Result	2/30	4/30	16/30	15/30	17/30	14/30	68/180

[a] In the ratios listed, numerator is number of plants infected; denominator, number of plants inoculated.

TABLE 6.—*Results* of *trials to determine the effect of post-infection feeding starvation on vector efficiency, using single green peach aphid apterae, single virus sources, a 0.5- to 3-min. infection feeding, a variable post-infection feeding starvation period, and a 1-hr. test feeding*

Environment[b] during starvation	Length of post-infection feeding starvation period in hours					Total	Unstarved check
	1	2	3	4	8		
Dry	3/30	0/30	0/30	0/30	1/30	4/150	9/30
Moist	4/30	0/30	1/30	0/30	0/30	5/150	

[a] In the ratios listed, numerator is number of plants infected; denominator, number of plants inoculated.

[b] The dry starved aphids were placed in a dry, filter paper-lined Petri dish; the moist starved aphids were placed in a moistened filter paper-lined Petri dish during the starvation period.

In testing this point, a replicated series of trials was made. The insects were starved prior to the infection feeding, given a 0.5- to 3-min. infection feeding, and then starved prior to being placed on the test plants for the following hourly intervals: 0, 1, 2, 3, 4, and 8 hr. The test feeding period was 1 hr. in duration. The results of the trials are given in table 6.

The results indicate that the initial rapid loss of the ability to transmit the virus under conditions of starvation is similar to those obtained by Watson (5). However, starvation beyond an hour resulted in almost complete loss of this ability. Watson starved the aphids under moist conditions, consequently table 6 includes aphids starved under dry conditions, and a comparable series starved under moist conditions, i.e., in a Petri dish lined completely with moistened filter paper. The results indicate that the two environments used had little effect upon the results.

Since the loss of ability to transmit the beet-mosaic virus was so rapid during the first hour, trials were made to determine during what portion of the hour the loss was occurring. In table 7 are found the results of trials using the intervals of 0, 15, 30, 45, and 60 min. of post-infection feeding starvation.

The test indicated that the loss of the virus was most pronounced in the first 15 min. of post-infection feeding starvation. Consequently the

TABLE 7.—*Results* of *trials to determine the effect of post-infection feeding starvation on vector efficiency, using single green peach aphid apterae, a single virus source, a 0.5- to 3-min. infection feeding, a variable post-infection feeding starvation period, and a 1-hr. test feeding*

	Length of post-infection feeding starvation period in minutes					Total
	0	15	30	45	60	
Result	13/30	6/30	2/30	1/30	0/30	22/150

[a] In the ratios listed, numerator is number of plants infected; denominator, number of plants inoculated.

next trial was made to test the intervals between 0 and 15 min. starvation. The intervals used in this test were: 0, 2, 5, 10, 15, and 60.   Table 8 lists the data obtained on these intervals.

The results indicate that loss of the ability to transmit the virus can begin within 2 min. of post-infection feeding starvation, but that starvation from 2 to 15 min. is fairly uniform in its action.

Briefly the data indicate, when viewed as a whole, that there is an initial loss of ability of the green peach aphid to transmit the beet-mosaic virus, following 2 min. of post-infection feeding starvation.   The degree of lost ability remains fairly constant for approximately 15 min , after which time it declines until about 1 hr. of starvation, at which time the loss is almost total.   An occasional individual, however, may retain the ability to trans-

TABLE 8.—*Results* of *trials to determine the effect of post-infection feeding starvation on vector efficiency, using single green peach aphid apterae, a single virus source, a 0.5- to 3-min. infection feeding, a variable post infection feeding starvation period, and a 1-hr. test feeding*

	Length of post-infection feeding starvation period in minutes						Total
	0	2	5	10	15	60	
Result	13/30	7/30	6/30	8/30	5/30	2/30	41/180

* In the ratios listed, numerator is number of plants infected; denominator, number of plants inoculated.

mit the virus for as long as 8 hr., under conditions of starvation following an infection feeding.

INFLUENCE OF THE NUMBER AND DURATION OF THE FEEDING PUNCTURES ON EFFICIENCY

Examination of the data which were obtained in the acquisition and inoculation threshold trials indicated that some factors were influencing the vector efficiency.   Since, in the acquisition threshold trial I and the inoculation threshold test, a common virus source, aphids from one population, and comparable test plants were used in simultaneously run replications, it was probably something associated with the test feeding period that determined the level of transmission.

A test was set up which compared the level of transmission obtained when 1 stylet insertion or puncture of a specified duration was made upon a test plant, with the level obtained when 5 punctures, each of the specified duration, were made upon a test plant.   The length of the individual test feedings was 10 sec.   Individuals permitted a single 10-sec. test feeding inoculated 8 out of 30 plants, while those allowed 5 separate 10-sec. test feedings on a single plant inoculated 23 out of 30 plants.   Thus the number of punctures made upon the test plant greatly influenced the transmission level.   This was also true with a persistent aphid-borne virus, *viz.*, the

sugar beet yellow-net virus (3). In the case of the beet-mosaic virus, the vector efficiency apparently can be doubled, or possibly tripled, by increasing the number of punctures from 1 to 5. From this it follows that the longer the test feeding period, the more potential punctures can be effected, and thus one explanation can be made for the effects of long test feeding periods upon increasing vector efficiency.

The results obtained in the inoculation threshold trials indicated that after 25 sec. of feeding, a slight increase in transmission followed an increase in feeding time (Table 2). In order to test this factor further, another series of trials was designed which compared the vector efficiency of insects which were allowed a 10-sec. test feeding, with those allowed a 60-sec. test feeding period. In these trials, the individuals which were allowed a single 10-sec. test feeding period inoculated 2 out of 30 plants, while those permitted a single 60-sec. puncture inoculated 6 out of 30 plants.

There is a strong indication that the longer the stylets remain *in situ,* the greater is the amount of transmission. Considering the results of both the trials made by varying the number, and increasing the duration, of the stylet insertion or puncture, it appears that both factors influence vector efficiency.

CONCLUSIONS

The experimental results have suggested the following conclusions as to the transmission of the beet-mosaic virus by the green peach aphid.

The acquisition and inoculation thresholds lie somewhere between 6 and 10 sec., with a mean transmission threshold value of approximately 1 min. The pre-infection feeding starvation period, to be effective, must be between 2 and 5 min. Aphids starving following an infection feeding begin to lose their ability to transmit the virus within 2 min., but the loss appears to be greater after 15 min. of starvation.

When an infective insect is fed on a healthy plant, both the number of punctures that are made, and the duration of any single puncture, can affect the chances for inoculation.

From these results, it is apparent that any attempt to stop the field spread of such a virus should consider at least the following points: the degree of infestation, the normal activity of the insects involved, the type of population, especially in reference to the number of alate, or winged, forms present, and the potential results of any control program upon population movement and general restlessness.

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STUDIES ON OAK WILT, CAUSED BY CHALARA QUERCINA[1]

ROY A. YOUNG[2]

Oak wilt is one of the most destructive tree diseases. Large oak trees may be killed within a few weeks after the first appearance of wilt symptoms. In 1942 the disease was shown to be caused by a fungus (16), which was later described and named *Chalara quercina* Henry (8). Little is known, however, about the manner in which this pathogen invades and destroys oak trees so rapidly. The studies reported in this paper were undertaken to determine the route of parasitic invasion within infected oak trees, the mechanism of wilting, the influence of environmental conditions on development of the parasite, and means of preventing spread of the disease in state parks and forests of Iowa.

ECONOMIC IMPORTANCE

During the past five years oak wilt has become increasingly important in the Upper Mississippi Valley. The wilt pathogen has been isolated from wilting oaks in Wisconsin, Minnesota, Iowa, and Illinois (10), and the disease has been observed generally over the State of Iowa (5), in residential districts of St. Louis (2), and in other localities in Missouri (3).

Wilt is generally recognized as the most serious problem in oak culture in Iowa. Although the areas of infestation enlarge slowly, large numbers of oaks are killed each year because of the tremendous number of infested areas. In farm woodlots in northeastern Iowa sufficient trees were killed in 1945 (7) to constitute almost 70 per cent of the potential annual red oak growth in those areas. Many oak trees are killed each year in woodlots, parks, and forests in southwestern Wisconsin, southeastern Minnesota, and Iowa.

Apparently most North American species of oak are susceptible to wilt. Natural infection has been observed in Wisconsin (10) or in Iowa (4, 5, 7, 13) on *Quercus alba, Q. borealis, Q. coccinea, Q. ellipsoidalis, Q. imbricaria, Q. macrocarpa, Q. marilandica, Q. muhlenbergii, Q. palustris, Q. stellata* and *Q. velutina.* Many additional species have been shown by greenhouse tests to be susceptible to wilt: *Q. prinus* (4), *Q. bicolor, Q. montana, Q. rubra, Q. rubra* var. *pagodaefolia, Q. shumardii* (5), *Q. falcata, Q. garryana, Q. hemisphaerica, Q. laevis, Q. phellos, Q. shumardii* var. *texana, Q. suber* (1), *Q. gambelii, Q. laurifolia, Q. nigra,* and *Q. virginiana* var. *maritima* (7). No varieties tested have been resistant.

The disease is most severe on trees of the red oak group (7, 9, 10). Large trees may be dead within 4 to 6 weeks after symptoms first appear. Typi-

cally, leaves in the crown of infected trees wilt, become bronze to brown, and fall from the tree. These symptoms appear progressively downward and inward throughout the tree. Red oaks infected in late summer or fall may put out leaves in the following spring, but develop typical symptoms and die within a few weeks. No red oak has ever been observed to recover from the disease. White and bur oaks are killed more slowly. Affected branches die, leaving many stagheads, but diseased trees may live for several years following initial infection.

No satisfactory method of controlling oak wilt has been found. Apparently the development of resistant varieties offers little promise. Host specificity does not seem to be an important factor, as limited tests showed that isolates from black, red, bur, and scarlet oak were pathogenic on black oak and an isolate from black was pathogenic on white oak (11).

Dietz and Barrett (6) reported that sanitary methods checked and sometimes controlled the spread of oak wilt. Tests (7) in which all infected oaks in an area and all healthy red oaks within a 50-foot perimeter were removed gave control of wilt. Results from plots in which only the dead and visibly diseased trees were removed were variable. In some plots spread was reduced, in others stopped. Pruning of infected branches was suggested as a means of saving white oaks with localized symptoms, although results were not perfect.

MATERIAL AND METHODS

Chalara quercina was isolated from naturally infected oak trees by the technique employed by Barrett (1) Sections of twigs, ½ inch or smaller, were surface-sterilized in a 20 per cent concentration of commercial Clorox for one minute and rinsed in sterile distilled water The twig sections were then placed on a glass slide which had been dipped in alcohol and flamed, and serial cross-sections were cut with a flamed razor blade and planted on potato-dextrose agar. Larger branches with smooth bark were surface sterilized by drenching an area with alcohol and flaming. The outer bark was removed with a flamed scalpel and sections consisting of inner bark, cambium, and one- and two-year-old wood were removed and planted on potato-dextrose agar. On large branches and boles, with rough, cracked bark, the bark was split away from the wood with a wood chisel and pieces of one- and two-year-old wood were removed with a flamed razor blade. In all methods of isolation seven sections of tissue were placed on edge in potato-dextrose agar and incubated for 5 to 10 days at 20° to 25° C. Transfers were made to potato-dextrose-agar slants and the cultures were further purified by single endospore isolation, employing the method of Keitt (12), immediately before making the tests.

A special method of isolation was used for studying the distribution of the fungus within the bole of the tree. One-inch cross sections were cut at 8- to 12-foot intervals up the bole. A radial strip 2 inches wide and extend-

ing the complete diameter of the cross-section was taken from each disk. With a circular saw these strips were sawed half-way through at the cambium, between the second and third growth rings and at one-inch intervals across the remaining distance. The partially sawed sections of wood were easily broken off by inserting a wood chisel in the saw path and exerting lateral pressure. A portion of wood was cut from each exposed surface and placed on potato-dextrose agar.

Diseased tissues were studied by histological methods. Sections of diseased leaves, petioles, and young stems were killed in Craf III or FAA and dehydrated with normal butyl alcohol (14). The tissues were infiltrated with paraffin, cast in Parlax, and sectioned with a rotary microtome. Older stems were killed in FAA and sectioned with a sliding microtome. Satisfactory staining was obtained when the sections were mordanted in an aqueous solution of 1 per cent potassium metabisulfite and 1 per cent tannic acid for 30 minutes, stained for 30 minutes in hemalum, and counter-stained for one hour in safranin. When stained in this manner mycelial walls were dark red and protoplasmic inclusions were dark red to purple.

The effect of cultural conditions on spore germination was determined by observing germination on 1 per cent dextrose agar. Spores were obtained by incubating cultures on potato-dextrose-agar slants for 10 days at 20° C. The spores were washed from the surface of the slant in sterile distilled water and adjusted to a concentration of 50,000 spores per ml. A droplet of spore suspension (0.1 ml.) was placed at each of four loci on 1 per cent dextrose agar in a Petri plate. Observations on spore germination were made at intervals between 16 and 96 hours by counting about 100 spores from each plate. The spores were not washed prior to the tests since they consistently germinated better than 90 per cent, and the agar surface was used in preference to a hanging droplet because secondary endospores are typically produced from the germ tubes of germinating spores and confuse the count in liquid media.

Virulence of the cultures was determined by inoculation of 2- to 4-year-old plants grown in the greenhouse. Successful inoculations were obtained either by making a longitudinal slit in the bark, inserting a weft of mycelium and spores and wrapping with moist cheesecloth; by spraying a conidial suspension onto the tree and making punctures or longitudinal cuts sufficiently deep to introduce spores into the outer xylem; or by hypodermically injecting conidial suspensions into the outer xylem. Inoculated plants were kept in a moist chamber for 48 hours, after which time they were transferred to a greenhouse bench. Symptoms typically appeared on apical leaves after 10 to 20 days. No successful inoculations were obtained without wounding the host.

Field inoculations were made by removing a small (¾ by 1 in.) rectangle of bark and filling the opening with a mass of agar, mycelium, and spores. The opening was then covered with moist cheesecloth.

EPIPHYTOLOGY

To obtain information on the methods of overwintering and natural spread of the pathogen, detailed observations were made on field plots established at Dolliver, Call, and Pike's Peak State Parks and in State Forest areas near McGregor, Iowa.

Chalara quercina lived over winter commonly on white and bur oaks. Persistence of the pathogen in these trees for several years was shown by isolation from infected trees over a period of three years. Overwintering was less common on red oaks since they were killed quickly. The fungus was isolated readily from roots, boles, branches, petioles, and leaves of infected trees. The pathogen persisted in stumps for two years after infected red and white oaks were removed. Sprouts which grew from such stumps usually developed typical wilt symptoms by midsummer.

In order to obtain information on field spread, plots were established in isolated areas where one or only a few trees were infected. Plots were
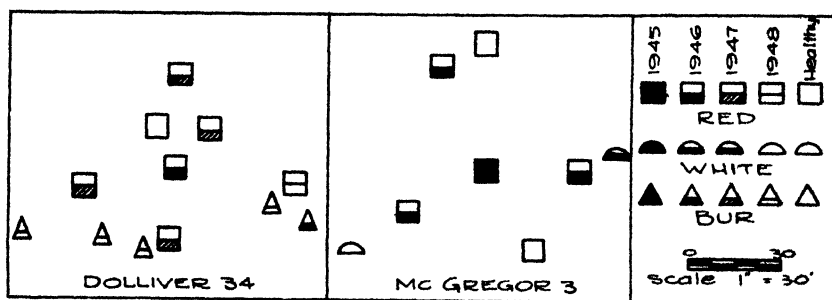
Fig. 1. Distribution of diseased trees in two localities within 3 years after initial infection. Each symbol represents one tree.

laid out with a rod and transit and maps were made of the location of all trees in each area. Records were taken periodically upon the amount of infection as determined by observation of symptoms and isolation of the pathogen

Typical spread patterns are shown in figure 1. In evenly distributed oak stands spread usually occurred in a roughly concentric pattern from the locus of infection outward to the nearest surrounding trees. The average distance of spread from diseased to healthy trees was about 30 to 40 feet. Usually no infection was observed beyond those trees immediately surrounding the first point of infection. However, all trees within the range of spread were not always involved. In areas where the disease had been active for several years it was possible to observe the same slow outward pattern of spread by the relative deterioration of dead oaks.

These data do not coincide with the typical pattern of spread by airborne spores (15) or insects (17, 18). If infection were by either of those agencies it would be expected that the incidence of infection would vary
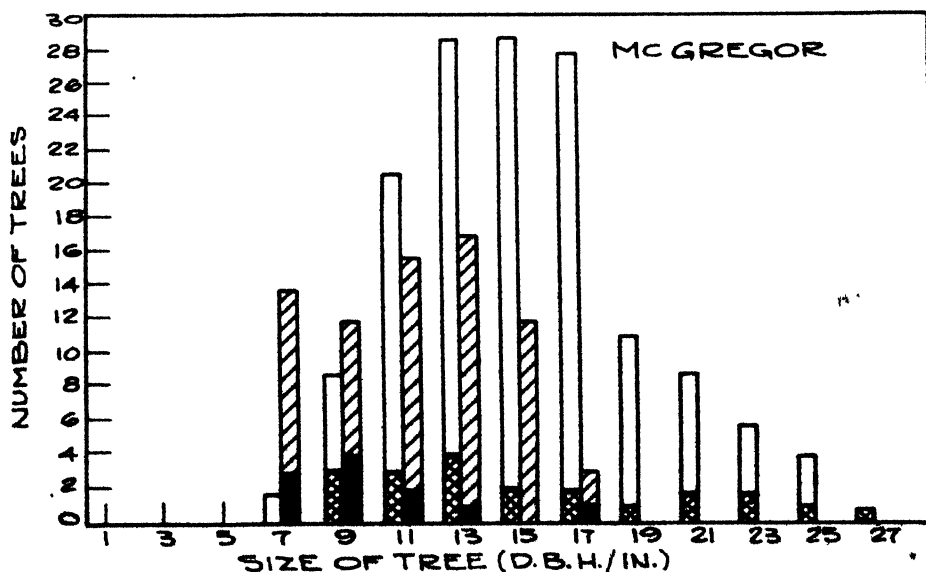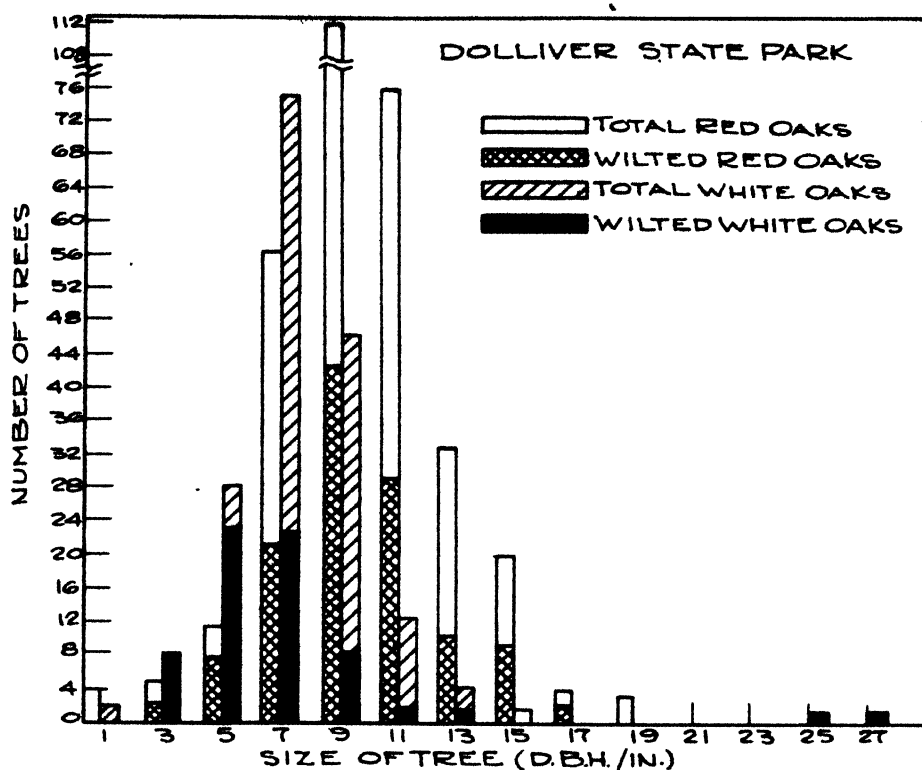
FIG. 2.   (Upper) Total number of red and white oaks at plot 1, Dolliver State Park, and number of wilted oaks in each class.   (Lower) Number of living red and white oaks of different sizes in plot 1, McGregor, in 1945, and number of trees in each class killed during 1946 and 1947.

inversely with the logarithm of the distance (18), and spread would be expected over a greater area than was observed.

Oak wilt is most obvious on large trees of the red oak group and it has been suggested that the large dominant trees in an area are the first attacked (5). To test for a correlation between size of tree and infection, complete surveys were made of all the oak trees in 8 wilt-infested areas. Typical data on the distribution of infection in two of these localities are given in figure 2. In general the number of infected trees of a given size was proportional to the total number of trees of that size in an area and the mean DBH (diameter at breast height) of the total population and of the wilted trees were almost identical. However, the mean DBH of the infected white oaks was slightly lower than the mean for the total white oak population. There is apparently no difference in the susceptibility of trees of different sizes to oak wilt.

To compare the incidence of natural infection of red and white oaks, data were tabulated on the percentage of the total volume of red and white

TABLE 1.—*Percentage of natural infection of red oak (Quercus borealis) and white oak (Q. alba) at four localities in northern Iowa, 1946–1947*

Location of plots	Red oak		White oak	
	Total no.	Percentage wilted	Total no.	Percentage wilted
Marquette Pt. I	60	10.0	89	6 7
Marquette Pt. III	39	17.9	41	2.4
Point Ann I	149	14 0	74	14.8
Dolliver I	90	11.1	50	12.0
Totals and averages	338	13 0	254	9.4

oaks infected in 1946–47 in areas in Dolliver State Park and near McGregor.

The data in table 1 show little difference in the susceptibility of red and white oaks to infection by *Chalara quercina* H. The extremely low figure for infection of white oaks in Marquette Point III is probably due to the unequal distribution of the species near the locus of infection in that plot.

At Pilot Knob State Park in north-central Iowa, dead and infected trees were so generally distributed throughout the park that study of spread from a single locus of infection was impossible. However, several areas were cruised and the number of dead and healthy trees of each oak species recorded (Table 2).

The percentages of the Hill's yellow (55.4) and red oak (52.9) trees that were dead were approximately twice as great as of white oak (28.3) and almost three times as great as of bur oak (19.9). However, large areas of dead bur oaks were found and numerous trees with a few dead branches were observed. These data bear out the observation that oak wilt is most destructive on species of the red oak group.

TABLE 2.—*Relative destruction of different species of oak at Pilot Knob State Park, Iowa, 1945*

Species		Total	Percentage dead
Q. macrocarpa (Bur)	—	2033	19.9
Q. alba (White)	—	300	28.3
Q. ellipsoidalis (Hill's yellow)		1688	55 4
Q. borealis (Red)	..	874	52.9
Total and average		4895	38 6

Records were taken on the time of first symptom development on trees in field plots at Dolliver State Park and McGregor, Iowa. The plots were checked at intervals of two weeks from May through September. During the summer of 1946 the greatest numbers of newly infected trees were recorded in June and July and a few were observed in May, August, and September (Table 3). In 1947, when the weather was subnormally cool

TABLE 3 —*Seasonal appearance of new wilt infection in field plots at Dolliver State Park and McGregor, Iowa*

Year of observation	Number of newly infected trees observed in				
	May	June	July	August	September
1946	5	30	29	5	5
1947	1	12	27	10	5

and heavy rains fell well into July, only a few trees had developed symptoms by the end of June and the peak of wilting was in July.

Host Specificity

Because of the wide variation in type of symptoms on different species of oak and the variation in rate of development in white oaks, tests were made for host specificity of different isolates   Cultures grown from single spores of isolates from six different species of *Quercus* were inoculated into

TABLE 4.—*Reaction of seven species of Quercus to Chalara quercina isolates from six different sources*

Species inoculated	Infection produced by culture from Quercus					
	borealis	alba	macrocarpa	ellipsoidalis	velutina	marilandica
Q. borealis	+	+	+	+	+	+
Q. ellipsoidalis	+	+	+	+	+	+
Q. palustris	+	+	+	+	+	+
Q. macrocarpa	+	+	+	+	+	+
Q. alba	+	+	+	+	+	+
Q. marilandica	+	+	0	+	+	+
Q. robur	+	+	0	+	+	+

+ = inoculation positive
0 = not tested

six healthy young trees of each of seven different species grown in the greenhouse. All isolates were pathogenic on all oak species tested (Table 4). The time required for symptom production and type of symptoms produced by each isolate was comparable. In addition to the species listed in table 4, numerous others were tested in the course of host range studies. No host specificity was observed in the different isolates.

DISTRIBUTION OF THE FUNGUS IN DISEASED TREES

The sudden and complete wilting of large oak trees suggests that *Chalara quercina* is a typical vascular parasite. If so, wilting might be induced by mechanical plugging of the vascular system, by production of a toxic substance which diffuses throughout the tree, or by rapid growth or transport of the pathogen from the site of infection throughout the tree. The distribution of the pathogen in five naturally infected red oaks was determined by host tissue isolations from all parts of the tree.

The pathogen was isolated from leaf midribs and petioles of wilting leaves, from branches and twigs bearing wilting leaves, and from the bole, but was not isolated from twigs on lower branches bearing apparently healthy leaves. The fungus was found well distributed perpendicularly in the bole but not always around the entire circumference. Radial distribution was restricted to the outer $\frac{1}{4}$ in. of sapwood. The fungus was never isolated from the heartwood.

Results of analyses of the distribution of the fungus within the tree by isolation were verified by study of microtome sections of diseased tissue from five red oaks and three white oaks. In these sections mycelium was observed in xylem vessels of leaf midribs, petioles, and twigs (Fig 3). Mycelium was apparently confined to the xylem and was not present in all vessels. Most of the hyphae grew longitudinally in the vessels. Mycelial development was sparse in general but in a few vessels many hyphae were observed. Numerous conidia were observed in the vessels (Fig. 3, d). These could be distinguished readily from excised hyphal tips and sections by the bipolar droplets which were present in the conidia.

These analyses show that the pathogen was usually present in wilted leaves and in twigs and stems bearing wilted leaves. Thus wilting is not necessarily due to a toxin transported from other locations in the tree nor to mechanical plugging. The presence of the small (2 to 3 × 5 to 7μ) conidia in vessels would provide a mechanism for rapid dissemination of the fungus throughout the tree in the transpiration stream. Vessels of both red and white oak, even those in leaf veins, are large enough to permit passage of the spores.

In tests in which spore suspensions were forced through 12-in. sections of red, white, and bur oak twigs under pressure, spores were present in the first drops of liquid to be drawn from the twigs. Numerous chains of 8 to 10 spores passed through the vessels intact.

EFFECT OF CULTURAL CONDITIONS ON DEVELOPMENT OF THE PATHOGEN

Petri dishes containing potato-dextrose agar were inoculated with 0.05 ml. of spore suspension containing about 250,000 spores per ml. and incubated at 25° C for 48 hours to allow equal germination in all plates.   Four
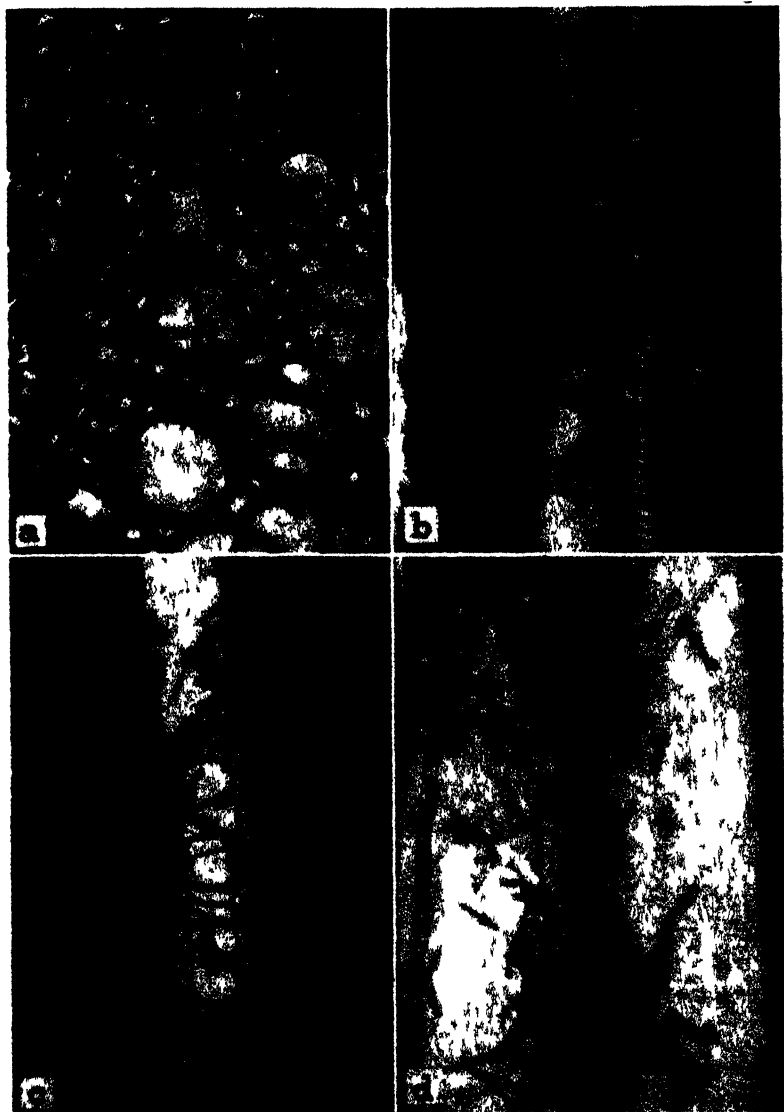
Fig. 3.   Sections of diseased red oak showing Chalara mycelium in vessels.   (317 x)
(a) Cross section of petiole.   (b, c, d) Longitudinal sections of current year's twig growth   Note endospores (arrows) in section d

plates were placed in each of seven incubators adjusted to 5°, 10°, 15°, 20°, 25°, 30°, and 35° C.   Daily growth increments were recorded by measuring the diameter of the colonies.

The most rapid growth (12 mm per day) occurred at 20° to 28° C. No growth occurred at 35° C , and at 5° C. none occurred until after 10 to 14 days Slow growth was recorded at 2° C. and 32° C Growth at 10°, 15°, and 20° C. was linear with time. However, at 25° and 30° C the curves showed a slowing of growth after the maximum rate was reached.
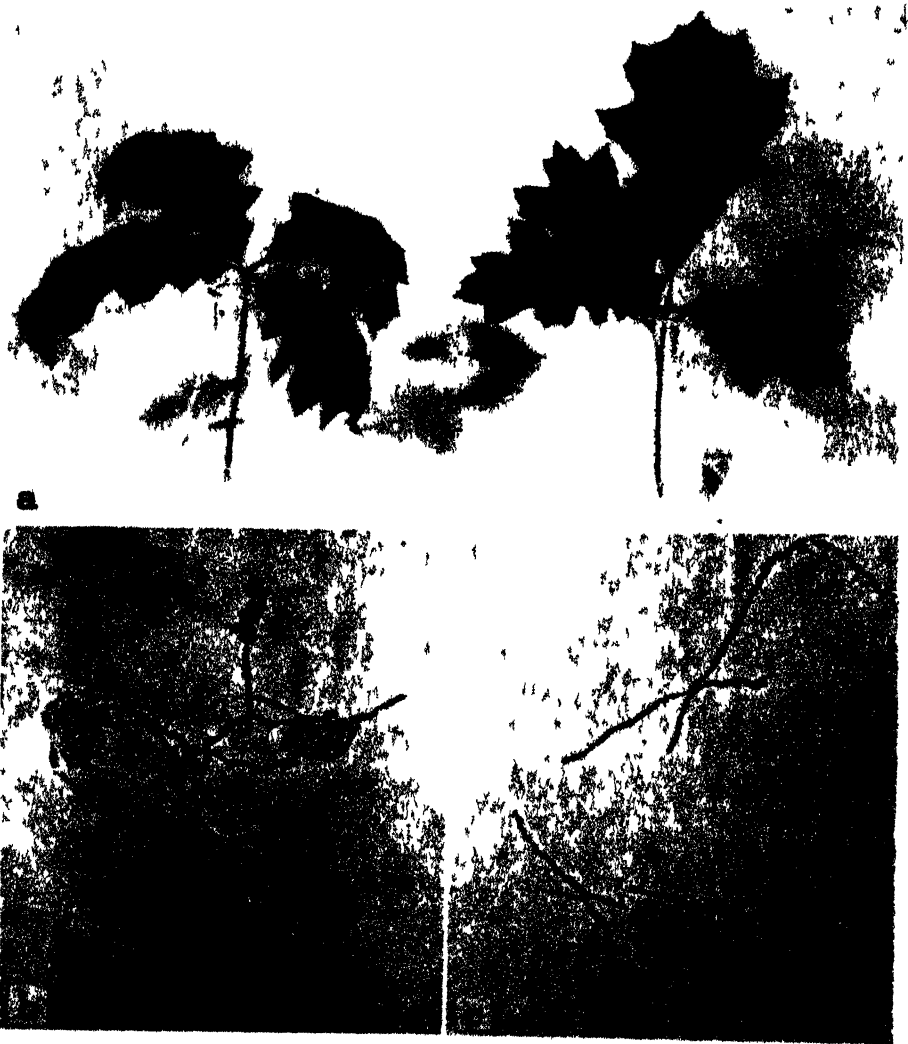
Fig 4 (a) Red oak cuttings after 24 hours in culture filtrate (left) and uninoculated medium filtrate (right) (b) Production of endospores after 48 hours at 25° C. and (c) at 30° C.

This inhibition was probably due to the accumulation of toxic by-products of fungus metabolism. Similar observations were made on growth in liquid culture. Cultures in yeast extract solution were autolysed after 90 days at 25° C. Cultures of the same age maintained at 20° C were still vigorous.

Spore germination tests were conducted over the same temperature range. Maximum spore germination occurred at 25° to 30° C. At this temperature 92 to 95 per cent of the spores germinated within 16 hours and up to 100 per cent after 24 hours. Germination was slow at the lower temperatures. At 10° C., 2 to 5 per cent germination was recorded after 96 hours. No germination occurred at 35° C. and spores which germinated at 32° C. did not continue to develop.

The method of germination differed markedly within the optimum temperature range. After 48 hours at 25° C. from 95 to 100 per cent of the germinating spores had produced numerous endospores (Fig. 4, b), the oldest of which had also germinated and were producing a second group of endospores. At 30° C. the majority of the germinating spores developed into vegetative hyphae and less than 15 per cent were producing endospores after 48 hours (Fig. 4, c). Endospores were produced abundantly after 48 hours at 20° C. and more slowly at 10° and 15° C. After 96 hours, spores had accumulated in large masses at 20° C. and 25° C. All spores that germinated at 15° C. were producing endospores, but at 30° C. endospore production was very limited.

Spore germination counts were made on plates of 2 per cent agar containing various concentrations of dextrose. Germination rose sharply from 0.0 to the 0.1 per cent level of sugar in the medium, with a range of better than 90 per cent at the 0.5 to 2 per cent sugar levels. From this point it tapered off to slightly better than 60 per cent at 4.0 per cent sugar. No higher concentrations were tested.

In addition to inhibiting spore germination, high concentrations of sugar were also unfavorable for the production of endogenous conidia by the germinating spores. The percentage of germinating spores that produced conidia from their germ tubes was lowest at the 4.0 per cent sugar level and increased as the sugar concentration decreased to 0.1 per cent. On the medium containing no sugar, sporulation was at a slightly lower level than on the 0.1 per cent medium.

Growth occurred over a range from pH 3.0 to pH 9.0 with the optimum from 5.0 to 7.0. The greatest amount of growth was recorded at 6.0. No growth occurred at pH 2 or pH 10.

MODE OF SYMPTOM INDUCTION

Spread of the pathogen through the host by movement of conidia in the transpiration stream, and induction of wilting by a metabolic product toxic to oaks, would explain the rapid progress of oak wilt through a tree. The rapid production of conidia by the pathogen and their movement through host vessels has been demonstrated.

To test for the production of metabolic products which would induce wilting, the fungus was grown in liquid culture on a modified Richards solution which contained asparagine and yeast extract. Culture filtrates

were obtained by passing the medium through a Büchner funnel and forcing the filtrate through a Seitz bacteriological filter to remove any *Chalara* particles or contaminating organisms. Dilutions were prepared by the addition of definite quantities of the filtrate to Erlenmeyer flasks containing measured amounts of sterile distilled water.

Oak cuttings placed in the filtrate reacted similarly to greenhouse-inoculated oaks. Severe wilting of young red oak cuttings was induced within 24 hours in a 50 per cent dilution of filtrate (Fig. 4, a). Wilting was general, with cupping and curling of leaves. After 70 hours large necrotic areas had developed between the veins. Bur and red oak cuttings showed slight symptoms of water loss after 24 hours, and after 120 hours were dry and papery and showed color zonation typical of that on leaves of greenhouse-inoculated plants.

The filtrates also were tested on 5- to 6-inch Bonny Best tomatoes. Plants were cut near the ground line with a razor blade, recut under water, and placed in the solution to be tested. In preliminary tests severe wilting of the cuttings was induced after 48 hours in 50 and 100 per cent concentrations of the filtrate. A small amount of drying of marginal lobes of leaflets was noted in control plants in noninoculated medium. Similar results were obtained using excised upper leaves from 10- to 12-inch plants.

To test for possible plugging of the lower stem, the tomato cuttings were removed from the filtrate after 48 hours, recut under water and placed in distilled water. None of the wilted plants recovered. Check plants treated similarly were still moderately turgid after 108 hours. Free-hand stem sections failed to show any indications of vascular plugging in the wilted plants. Bacteria were numerous in stems of the check plants and both cortical and vascular necrosis more extensive. It was also noted that control cuttings developed roots abundantly when recut and transferred to distilled water. Roots did not develop on the cuttings that had been exposed to the filtrate.

Dilutions of the filtrate ranging from 50 per cent down to 3 per cent were tested for toxicity to tomato cuttings. Severe wilting was induced within 48 hours in concentrations down to 12.5 per cent and slight marginal chlorosis and drying of leaflets was induced at 3 per cent concentration. Wilting was induced with filtrates from cultures varying in age from 15 to 90 days.

CONTROL

Although sanitation is usually more effective in control of canker diseases of trees than of wilts, studies were undertaken to determine whether infested areas could be disinfested by removal of diseased trees and infected white oak trees could be saved by removal of diseased branches. A series of plots established by Dr. S. M. Dietz was carefully surveyed for infection and records were taken on the progress of the disease before and after removal of diseased members.

Eradication experiments of three types were conducted. (1) In one area of heavy infestation all dead and diseased oaks and all living oaks within a 50-foot perimeter were cut in 1944. All branches, small twigs, and leaves were piled and burned and the stumps were stripped of bark. Logs and large limbs were sawed and used for firewood. No further spread was observed in this plot through 1947.

(2) In a second plot all dead and diseased trees and all living red oaks within a 50-foot perimeter were cut and disposed of as above. Living white and bur oaks were not removed from the plot. Trees continued to become infected in this area and were killed in large numbers in 1947.

(3) Two groups of plots separated by a valley one-half to three quarters of a mile wide were conveniently situated for a field comparison of the effect of eradication on incidence of infection, and were handled as follows. Spread was allowed to occur naturally in the 13 plots on one side of the valley from 1944 through the summer of 1945. In the winter of 1945–46 all dead and diseased trees were removed. A reduction in the number of infected trees of more than 50 per cent was observed in these plots during the summers of 1946 and 1947. In the second group of plots spread was allowed to occur without interference through the summer of 1946. The incidence of infection did not decrease in 1946. Following the removal of all dead and dying trees during November and December of 1946, however, a reduction of almost 40 per cent in the incidence of infection was noted in 1947.

From these data it appears that oak wilt may be controlled by thorough eradication of dead and diseased oaks in an area, provided a large enough perimeter of apparently healthy trees be removed from around the center of infection to eliminate any infected trees not showing symptoms. Failure to eradicate the pathogen from plot 2, and subsequent spread, were probably due to mildly infected white oak trees which went unnoticed in the removal of diseased trees. In all cases the yearly incidence of infection was decreased by removal of dead and diseased trees from infested areas. However, complete eradication was accomplished only in those areas in which a 50-foot perimeter of healthy trees was also removed.

The general distribution of the pathogen in infected red oaks precludes the use of pruning as a means of saving diseased trees of that group. However, oak wilt progresses slowly in white and bur oaks and apparently often is confined locally within the tree. If the latter were true, removal of infected portions might offer a satisfactory means of saving infected white oak trees. When the distribution of symptoms was favorable on naturally infected white oaks in field plots, diseased portions of the tree were removed at points 4 to 6 feet back of the innermost symptoms. In a few cases a portion amounting to as much as 15 to 20 feet of the tops of white oaks was removed.

The results of pruning were variable (Table 5). Apparently the organ-

ism was successfully eradicated from tree 7 by removal of four branches bearing wilted leaves. The remaining trees reacted variously in regard to recurrence of symptoms. Some developed general symptoms shortly after pruning. Others had no symptoms for over a year, then developed general symptoms.

TABLE 5.—*Effectiveness of pruning as a means of eradicating Chalara quercina from naturally infected white oaks*

Number and diameter of tree	Date of first pruning	Date symptoms recurred	Chalara reisolated	Remarks
1. 6–in.	June 20, 1945	July 15, 1945	Yes	General symptoms Cut July 15, 1945
2. 9–in.	do	July 18, 1946	No	
		June 13, 1948	Yes	Cut June 16, 1948
3. 11–in.	do	June 20, 1946	Yes	
		July 18, 1946	Yes	General symptoms
		July 12, 1947	Yes	Tree cut July 12, 1947
4. 9–in.	do	June 19, 1946	Yes	
		Sept. 18, 1947	Yes	Single branch pruned
		June 13, 1948	Yes	Single branch pruned
5. 7–in.[a]	June 21, 1946	July 12, 1947	Yes	General symptoms Cut July 12, 1947
6. 10–in.	July 10, 1946	July 12, 1947	Yes	General symptoms Cut July 12, 1947
7. 10–in.[a]	July 25, 1946	No symptoms	No	No further symptoms
8. 10–in.	July 13, 1946	July 27, 1946	Yes	General symptoms Cut July 27, 1946
9. 12–in.	July 11, 1946	Sept. 15, 1946	Yes	Pruned again
		June 20, 1948		General symptoms

[a] Symptoms scattered over tree. All branches with symptoms were pruned.

DISCUSSION

In the course of these investigations it has been observed that the oak wilt pathogen may live overwinter in infected trees and stumps. Trees of the white oak group may retain perennial infection for several years and red oaks may often harbor the pathogen for as long as a year. Definite proof has been offered that fungus mycelium and conidia develop in xylem vessels of these hosts. The manner in which hyphae or conidia leave infected plants and are transferred to healthy plants is not known. It is entirely possible that the fungus may have a perfect stage, as yet undiscovered, which would further supplement these known reservoirs of inoculum or which may be the chief means of dissemination.

Greenhouse studies indicate that the fungus cannot establish itself in uninjured stem or leaf tissue. No successful inoculations were obtained without first providing a wound entrance for the pathogen. Once established in the host, however, the organism spreads rapidly throughout the tree. The abundant production of small conidia provides a means of rapid spread in the transpiration stream. Conidia are produced most abundantly

at 20° to 28° C., a temperature range that prevails during the greater part of each summer day in June, July, and August in this region. Conidial production begins almost immediately following spore germination in culture and continues at a rapid rate as the fungus develops. Conidia may be found generally distributed in xylem vessels (Fig. 3, d) of infected trees.

Considering temperature as the chief factor limiting sporulation, the most favorable time for rapid spread of the organism would be in the latter half of June and first half of July when night temperatures are at a level permitting growth, and daytime temperatures seldom exceed the upper limits of the optimum range. Data on time of infection as based on development of symptoms bear out this hypothesis (Table 3).

The fact that conidia were readily passed through oak twigs under suction would indicate that they may be carried in the transpiration stream. The vessels of white oaks are smaller than those of red oaks, and more occluded with tyloses. Therefore, it is possible that these anatomical features may impede the movement of Chalara spores in white oak and in part contribute to the slower, more restricted, invasion of this type of oak.

The pathogen, when grown in liquid culture, produces certain metabolic products that are chemically stable and capable of inducing wilt symptoms typical of those associated with the disease. These toxic substances are not specific for oak since they also induce wilting of tomato cuttings in a similar fashion. The symptoms produced by the toxic products are so similar to the ordinary symptoms associated with oak wilt that there can be little doubt that the toxic substances play an important role in disease production. Red, white, and bur oak cuttings all reacted to the filtrate to produce symptoms typical of greenhouse-inoculated oaks. It is reasonable to assume, therefore, that the resistance in white oaks may be more appropriately attributed to restriction of invasion and growth of the fungus than to specificity of or immunity from the toxin.

Control of a tree disease such as oak wilt presents a tremendous problem. The sanitary measures which provided satisfactory disease control were too drastic to be of practical value for large-scale control efforts. Pruning of mildly infected white oaks may offer a means of saving individual trees. Uniformly good results from pruning elms with Dutch elm disease have been reported (18). Successful eradication of the pathogen from white oaks by pruning would depend on the reliability of symptoms as an indication of the extent of the pathogen's distribution within the tree. Preliminary results indicate that the organism is often restricted to the region of symptoms in white oaks.

SUMMARY

Chalara quercina was observed to overwinter commonly in white and bur oak trees, in stumps of diseased trees that had been removed, and occasionally in red oak trees that were infected late in the season.

The disease spread in a pattern not typical of windblown spore or insect dissemination. Oak wilt usually spread from an infection locus to the nearest healthy trees.

Data on percentage infection of red and white oak species showed little difference in susceptibility to infection. However, data on destruction of species of oak showed much greater losses in trees of the red oak group than in trees of the white oak group. No difference was observed in the incidence of infection of different sizes of trees. The number of infected trees of a given size was proportional to the total number of trees of that size in an area.

No host specificity was observed in cross-inoculation tests with isolates from different species of oaks. Isolates from each species produced typical disease symptoms when inoculated into other species.

Chalara quercina was isolated from all parts of diseased trees except the acorn. In histological preparations the fungus was observed in xylem vessels of leaf midribs, petioles, and stems. Conidia were observed in host tracheae, and conidia in water suspension passed readily through red and white oak stem sections.

Isolates of *Chalara quercina* grew well over a range from 16° to 28° C. with most rapid growth at 22° to 26° C. The optimum temperature range for spore germination was 25° to 30° C. At 25° C. endogenous conidia were typically produced from germ tubes of all germinating spores, while at 30° C. more than 85 per cent of the spores formed vegetative hyphae on germination.

The pathogen grew well on all complete nutrient media tested but grew best on potato-dextrose agar and oatmeal agar. Spores germinated best at 1 per cent dextrose concentration. Spore production from germinating spores tended to increase as the sugar concentration was decreased from 4 per cent to 0.1 per cent. The optimum pH range for growth was from pH 5 to pH 7 with limits at pH 3 and pH 9.

A metabolic product, which readily induced wilting of tomato and oak cuttings, was produced by *Chalara quercina* when grown on a modified Richards solution containing asparagine and yeast extract. Symptoms on toxin-wilted oak cuttings were typical of those produced on diseased greenhouse oaks.

Eradication experiments showed that the pathogen could be removed from an area by extreme sanitary measures. Incidence of infection was reduced by removal of diseased trees from areas of infection.

Results from pruning experiments were inconsistent but indicated that white oaks might often be saved by removal of diseased parts soon after the appearance of symptoms. Diseased branches were pruned at a point 4 to 6 feet back of the symptoms.

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PARASITIC ACTION OF STREPTOMYCES SCABIES ON ROOTS OF SEEDLINGS[1]

W. J. H o o k e r [2]

INTRODUCTION

A sound approach to potato scab control by the use of suitable crop rotation is dependent, at least in part, on an understanding of the host range of the potato scab organism, *Streptomyces scabies* (Thaxt.) Waksman and Henrici.[3] Heretofore little attention has been given to the parasitism by *S. scabies* on the fibrous roots of various field crops, although infection of the roots of potato has been well established (4, 8, 13, 14). KenKnight (11) reviewed the literature describing scablike lesions on the underground organs of a large group of plants including beet, cabbage, turnip, rutabaga, radish, parsnip, and carrot. He reported as additional hosts, *Amaranthus retroflexus, Solanum nigrum,* and *Solanum melongena.*

The research herein reported was undertaken to determine the host range of *Streptomyces scabies* by investigating infection on the fibrous roots of various plants grown under a number of cultural conditions. It has been reported (6) from this laboratory that seedlings of various plants were unable to survive in peat soil artificially infested with *S. scabies.* Additional progress reports (5, 7) concerning certain phases of the work have been made.

EXPERIMENTAL PROCEDURE

Seed of plants to be tested for resistance to *Streptomyces scabies* were surface disinfected in 25 per cent commercial Clorox (5.25 per cent sodium hypochlorite) for 15 min.[4], rinsed in tap water, and germinated on sterile moist filter paper in Petri plates. When the radicles were approximately 1 cm. long, plants were transferred to the respective media for growth.

In the early trials, sterilized peat soil contained in ⅜-in. Pyrex test tubes which were either 8 or 12 in. long was artificially infested with cultures of *Streptomyces scabies.* After approximately 1 week of incubation, a germinated seed was planted in the soil of the tube and the cotton plug was replaced. Controls containing sterilized, noninfested soil were maintained. In other trials, seeds were planted directly into pots containing sterilized peat soil which had been artificially infested.

Root infection in quartz sand cultures was obtained by mixing a suspension of spores and aerial hyphae from a culture of *Streptomyces* sp.

[3] Binomials of microorganisms used by Breed *et al.* (3) are employed.

[4] Although this treatment reduced the number of microorganisms on the surface, bacterial contaminants were often demonstrated when seedlings were planted on potato-dextrose agar.

with sterilized sand contained in either 4-in. pots or 250-ml. beakers
equipped with suitable drainage tubes. Germinated seed were covered
with infested sand and the sand was kept moist with tap water. After
approximately 10 days' growth, plants were watered with a modified Hoag-
land's solution (20).

Inoculum was grown on modified potato-dextrose agar (19) in 9-cm.
Petri plates. It consisted of a suspension of aerial hyphae and spores from
⅛ to ¼ of a Petri dish culture, and was poured into a covered sterile 100-ml.
beaker. This inoculum was mixed with sterile, melted (37°–40° C.) soil
agar prepared by extracting the water-soluble material from 25 gm. of soil
in 1 liter of water and solidifying the filtrate with 1.3 per cent agar. After
the infested medium had solidified, radicles of germinated seed were in-
serted. Lids were removed in a day or two to permit growth of aerial
portions, and tap water was used for watering.

Plants were grown at room temperatures (20° to 25° C.) and under
continuous illumination. After the growth period, roots were readily
washed free from the agar and fresh root weights were recorded. Pre-
liminary trials with wheat and pea had shown that differences in fresh
root weights were similar to those obtained with dry root weights and, be-
cause of ease of preparation, the former were used.

Four cultures of *Streptomyces scabies* were compared with six of *Strep-
tomyces* sp. which were incapable of causing scab on tubers of Cobbler po-
tato (*Solanum tuberosum* L.). A brief description of these ten cultures
is given in table 1. The cultures were tested on soybean (*Glycine max*
Merr. var. Lincoln), pea (*Pisum sativum* L. var. Little Marvel), wheat
(*Triticum vulgare* Vill. var. Cadet Spring), radish (*Raphanus sativus* L.
var. Early Scarlet Globe), garden beet (*Beta vulgaris* L. var. Detroit Per-
fected Strain), corn (*Zea mays* L. inbred Hy), and cucumber (*Cucumis
sativus* L. var. National Pickling).

For histological studies, plants were grown in 2 per cent soil agar.
Tissues were killed in Navaschin's Craf III solution (18), dehydrated in
dioxan and N-butyl alcohol, imbedded in paraffin and cut at 15 μ. Sections
were stained in hematoxylin for 12–16 hr. preceded by 4–8 hr. in iron-alum
mordant. Although results with hematoxylin were very satisfactory, saf-
ranin was occasionally used as a counter stain.

INFECTION IN ARTIFICIALLY INFESTED PEAT SOIL

Seedling plants grown from surface disinfected seed were transplanted
to large test tubes containing sterilized peat soil infested with *Strepto-
myces scabies*. Infection of aerial parts was common, especially in cu-
cumber with aerial *Streptomyces* filaments present on either the cotyledons
or growing point. Other plants responded by a general collapse of aerial
portions accompanied by severe root necrosis (Fig. 1). Plants grown in
the presence of *S. scabies* died prematurely, whereas those grown in steri-
lized, noninfested peat survived to the end of the observation period·(Table

TABLE 1.—*Source and certain characteristics of the Streptomyces culture tested*

Culture number and name	Source of isolate[a]	Ability to scab Cobbler potato	Growth on soil agar	Color of surface growth on modified potato-dextrose agar[b]	Color of surface ring in separated milk[b]
1. *Streptomyces lavendulae* (Waksman and Curtis) Waksman and Henrici	S. A. Waksman	none	trace	pale mouse gray	Brussels brown
2. *S. sp.*	C. H. Meredith (culture 216)	none	slight	pale purplish gray	clear
3. *S. sp.*	shallow scab lesion on potato tuber	none	slight	white	clear
4. *S. scabies*	do	moderate	trace	pale gull gray	Brussels brown
5. *S. scabies*	deep scab lesion on root of red beet	marked	trace	pallid purplish gray	Brussels brown
6. *S. sp.*	do	none	slight	light gull gray	clear
7. *S. scabies*	do	marked	trace	pallid purplish gray	Sudan brown
8. *S. sp.*	apparently scab free skin of potato tuber	none	slight	pallid neutral gray	Brussels brown
9. *S. scabies*	shallow scab lesion on potato tuber	marked	slight	drab gray	Brussels brown
10. *S. sp.*	do	none	slight	white	clear

[a] Cultures 3, 4, 8, 9, and 10 were isolated in February, 1944, and cultures 5, 6, and 7, in September, 1944, from plants grown in peat soil at Crystal Lake, Iowa.
[b] Colors according to Ridgeway (16).
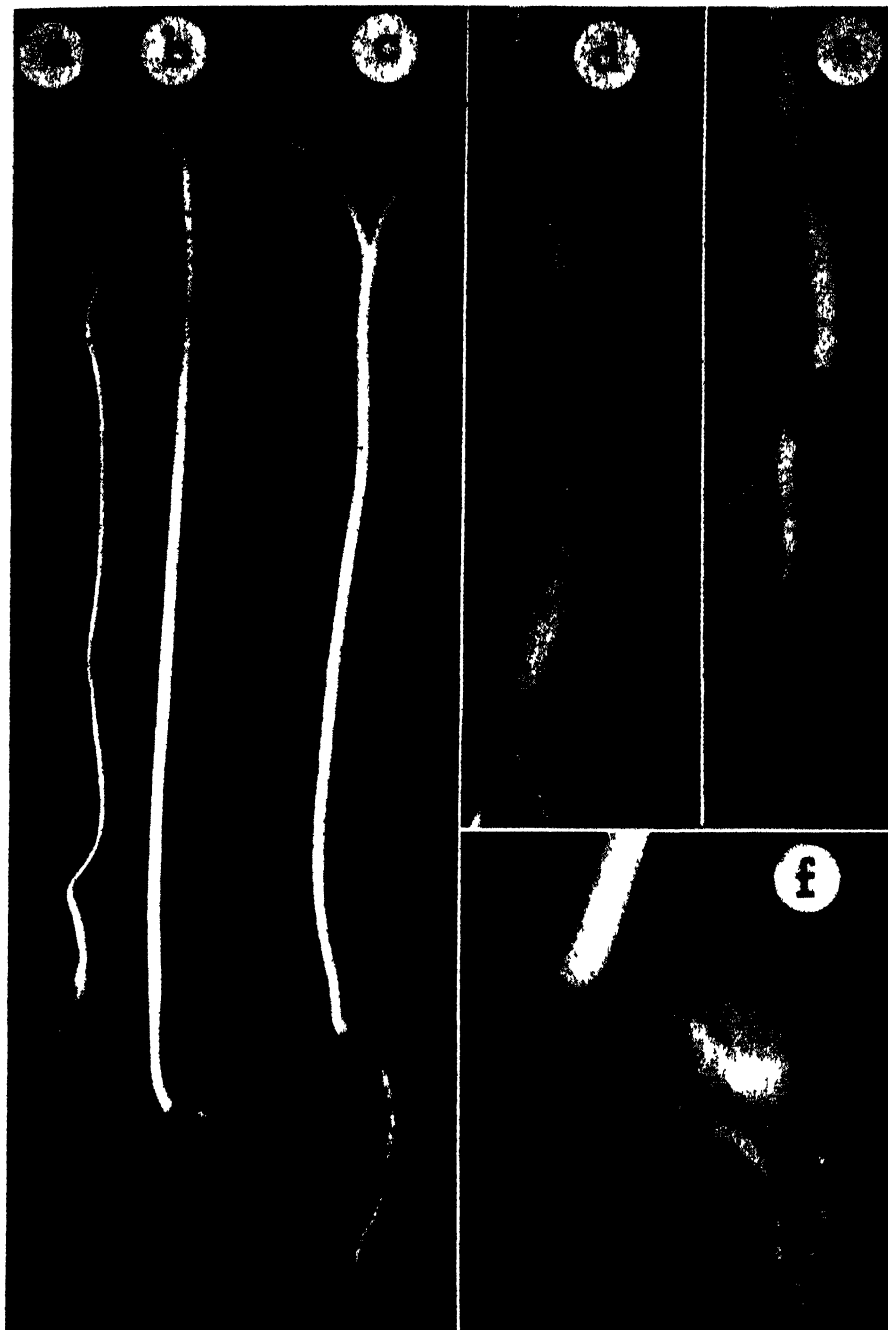
FIG. 1.   a, b, c. Radish seedlings grown 16 days in tubes containing peat soil, (a, b) infested with *Streptomyces scabies*, and (c) noninfested.   d, e. Lesions on soybean grown in pots containing peat soil infested with *S. scabies*.   f. Lesion on pea grown under similar conditions.   *Streptomyces* filaments were demonstrated in lesions of d, e, and f. (Photographs by John Stabe.)

TABLE 2.—*Survival of seedlings grown in large test tubes containing sterilized peat soil artificially infested with Streptomyces scabies*

Test plant	Period of growth	Infested peat[a]		
		Healthy	Diseased	Dead
	Days	*Number*	*Number*	*Number*
Radish, Scarlet Globe	19	0	2	11
	14	0	1	4
Turnip, Purple Top White Globe	22	0	3	3
	22	0	1	3
Cabbage, Improved Wis. Hollander	30	0	0	5
Spinach, Nobel Giant	11	0	2	4
	15	0	4	2
Cucumber, Early Pickling	18	1	7	2
	23	0	4	3
Potato, 116–13, open pollinated seed	19	0	1	5

[a] Equal numbers of control plants in noninfested peat remained healthy in each case.

2). In spite of the precautions taken to develop plants under aseptic conditions, it was in only rare instances that bacterial contaminants were not demonstrated when transfers were made at the end of the growth period to potato-dextrose agar. Although *S. scabies* was not in pure culture, the reaction was consistent with plants developing well in the noninfested controls and with necrosis and premature death where *S. scabies* was present.

Attempts to develop infection in open pots in the greenhouse were generally unsatisfactory, although scablike lesions were occasionally obtained on the lower stems of garden pea and soybean plants grown in infested peat (Fig. 1). In these lesions, filaments of *Streptomyces scabies* were demonstrated histologically in the tissues and spores and aerial filaments were present on the surface of lesions.

INFECTION IN QUARTZ SAND

Surface disinfected wheat seed were planted in sterilized, infested

TABLE 3.—*Necrosis of wheat root tips in quartz sand artificially infested with Streptomycetes*

Culture number	Pathogenicity to potato	Experiment 1				Experiment 2	
		13 days' growth		23 days' growth		23 days' growth	
		Roots[a]	Necrotic at tip	Roots[a]	Necrotic at tip	Roots[b]	Necrotic at tip
		Number	*Number*	*Number*	*Number*	*Number*	*Number*
4	moderate	39	9	40	12	38.2	26.2
5	severe	40	17	38	30		
9	severe	44	17	41	36	34.5	31.7
6	none	36	0	42	0	34.3	0.3
none		45	0	39	2	37.2	0.2

[a] Figures indicate the total number of seminal roots on 10 seedlings.
[b] Figures represent the average of 4 replications of 10 seeds each.

quartz sand. After 2 to 3 weeks, symptoms on wheat consisted of an orange-yellow to light brown necrosis of the root tips with generally no macroscopically visible discoloration of the cortex. The extent of root tip necrosis in the first trial (Table 3) became progressively more severe from 13 days to 23 days with cultures 4, 5, and 9. In a more extensive test, the extent of lateral branching was slightly reduced with cultures 4 and 5, pathogenic to potato, and root tip necrosis was similar to that previously obtained. In this second trial there was only a trace of root tip discoloration, the cause of which could not be determined, in the water controls and with culture 6.

Quantitative measurements of root necrosis with soybean were difficult since a light tan cortical discoloration was present over much of the root

TABLE 4.—*Growth of seedling plants in soil agar infested with Streptomyces sp. cultures*

Culture number and species		Weight of roots grown in infested soil agar[a]						
		Soybean	Pea	Wheat	Corn	Cucumber	Radish	Garden beet
		mg.	mg.	mg.	mg.	mg.	mg.	mg.
4.	*Streptomyces scabies*	152	90	28	279	73	38	4.8
5.	do	109	97	16	268	84	31	5.3
7.	do	104	89	18	250	82	22	6.3
9.	do	131	97	12	263	92	27	6.6
Av.		124	93	18	265	83	30	5.7
1.	*S. lavendulae*	268	363	34	343	99	49	9.1
2.	*S. sp.*	309	355	47	396	100	67	9.6
3.	do	344	334	45	416	97	52	7.1
6.	do	392	373	45	466	95	46	7.2
8.	do	273	270	51	344	81	53	8.6
10.	do	253	333	46	366	80	56	8.0
Av.		290	339	45	389	92	54	8.3
Water control		318	353	51	381	78	56	9.0
	do	306		49	386	80	66	
Av.		312	353	50	384	79	61	9.0

[a] Figures represent the average fresh weight of roots from at least 3 beakers each containing approximately 5 plants of soybean, wheat, radish, and beet, and 3 of pea, corn, and cucumber. Growth period, 16 days except 10 days with soybean and 18 days with corn and cucumber.

area as well as at the tips. In type it was similar to that observed later in the soil agar, but it was not so consistently severe.

INFECTION IN SOIL AGAR

In December, 1945, experiments were begun using soil agar as a medium for plant growth. Such a medium permitted observation of roots during the growth period and supported only meager growth of the streptomycetes under observation (Table 1). Contaminants developed only sparsely in it.

Growth responses of soybean, pea, wheat, corn, cucumber, radish, and

garden beet to the ten cultures in soil agar are shown in table 4. Each of the plants listed was tested at least three times, and average root weights are presented from one representative trial consisting of at least three similar beakers, each containing equivalent numbers of plants. With all plants except cucumber, cultures fell into two distinct groups when separation was made either on the basis of root weight or of root necrosis. *Streptomyces scabies* cultures 4, 5, 7, and 9, known to cause scab on potato, reduced the root growth and severely impaired the development of laterals. Root necrosis was severe, especially at the tips and at the bases of the secondary roots. Root growth with cultures 1, 2, 3, 6, 8, and 10, known to be nonparasitic to potato, was neither appreciably reduced nor different from that developing in the sterile soil agar controls. Cucumbers had considerable resistance to *S. scabies* with little root weight reduction at the end of 18 days' growth.

Soybean and pea. These plants exposed to the four cultures of *Streptomyces scabies* had similar symptoms, generally becoming evident in 6 days, characterized by severe necrosis of the root tips and a severe general cortical necrosis of both the primary and lateral roots, with the latter being either absent or very short (Fig. 2). Fresh root weights (Table 4) with *S. scabies* were less than one-half those of roots grown in the water controls or with cultures nonparasitic to potato. Roots exposed to cultures nonparasitic to potato were white, well branched, and generally indistinguishable from those of plants grown in the water controls. Although there was some variation in root weights of these plants within the group of cultures nonparasitic to potato as well as within the group of *S. scabies* cultures, no definite trend was evident in subsequent trials. There was no overlapping of either root weights or necrotic reaction between cultures of either group. Aerial portions of soybean and pea were usually stunted, reflecting the severely necrotic condition of the roots. In certain instances aerial portions were slightly swollen, suggesting systemic invasion by *S. scabies*. This reaction was especially common when sprouted seeds were planted somewhat too deeply in the agar.

Henderson's bush Lima bean (*Phaseolus lunatus* L.) was similar in reaction to soybean and pea.

Small grains. Cultures of *Streptomyces scabies* caused necrosis of wheat root tips which was usually evident by the sixth day as a light tan discoloration, later changing to various shades of yellow to orange and light brown, and in advanced cases to dark brown. Generally there was little to no discoloration of the root cortex away from either the tip or points of origin of lateral roots (Fig. 3). After a root tip had become necrotic no further elongation was observed. Lateral roots generally failed to emerge, but their position was evident as punctate necrotic spots located at regular intervals along the roots. It was demonstrated histologically that these root tips had been almost replaced by a mass of streptomycetous filaments. Fresh root weights with *S. scabies* (Table 4) were less than one-

half those of plants grown in the controls or with the cultures nonparasitic
to potato.
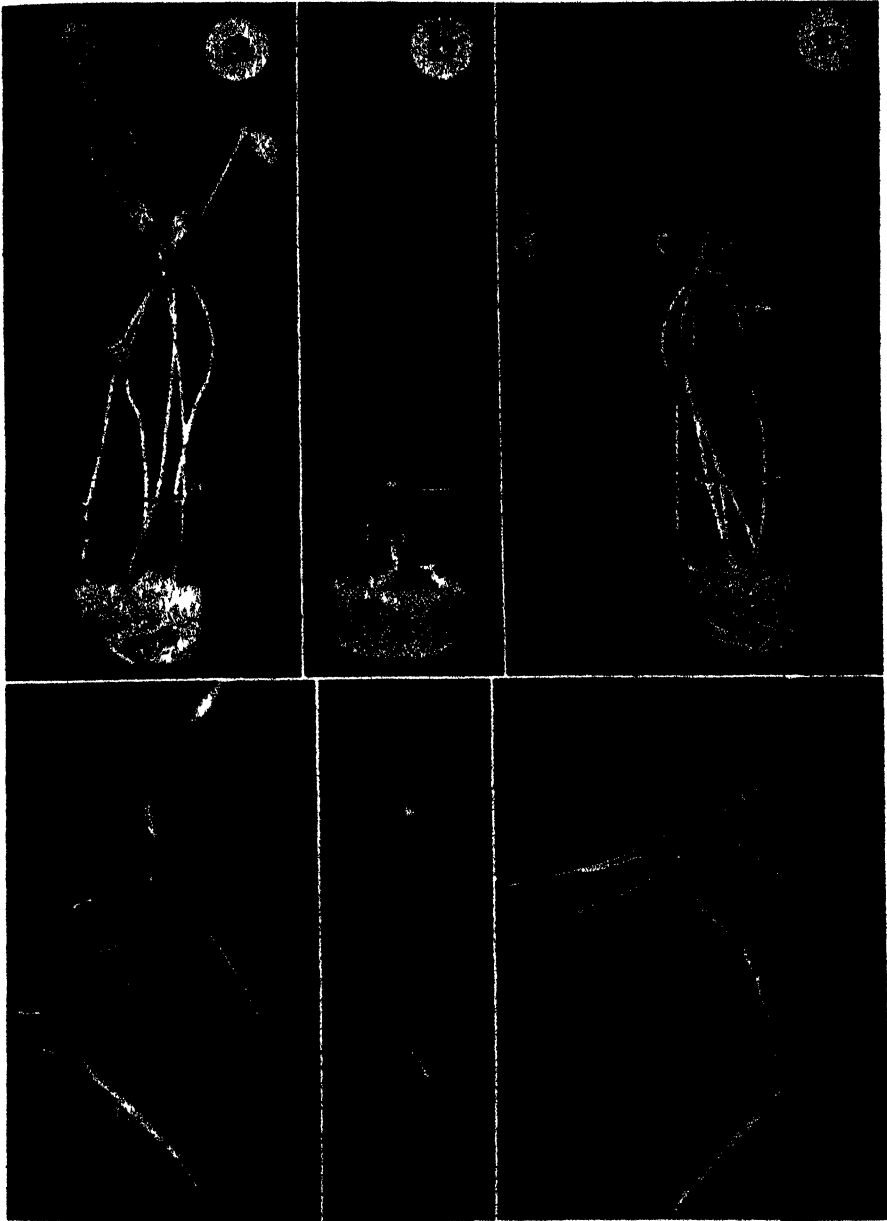
FIG. 2. Soybeans and representative roots grown 10 days in beakers containing soil
agar: a, infested with culture 6 of *Streptomyces* sp.; b, infested with culture 7 of *S.
scabies*; c, water control.

Root growth of control plants in the agar medium alone was generally
good with lateral roots and root hairs present by the fifth day and with no
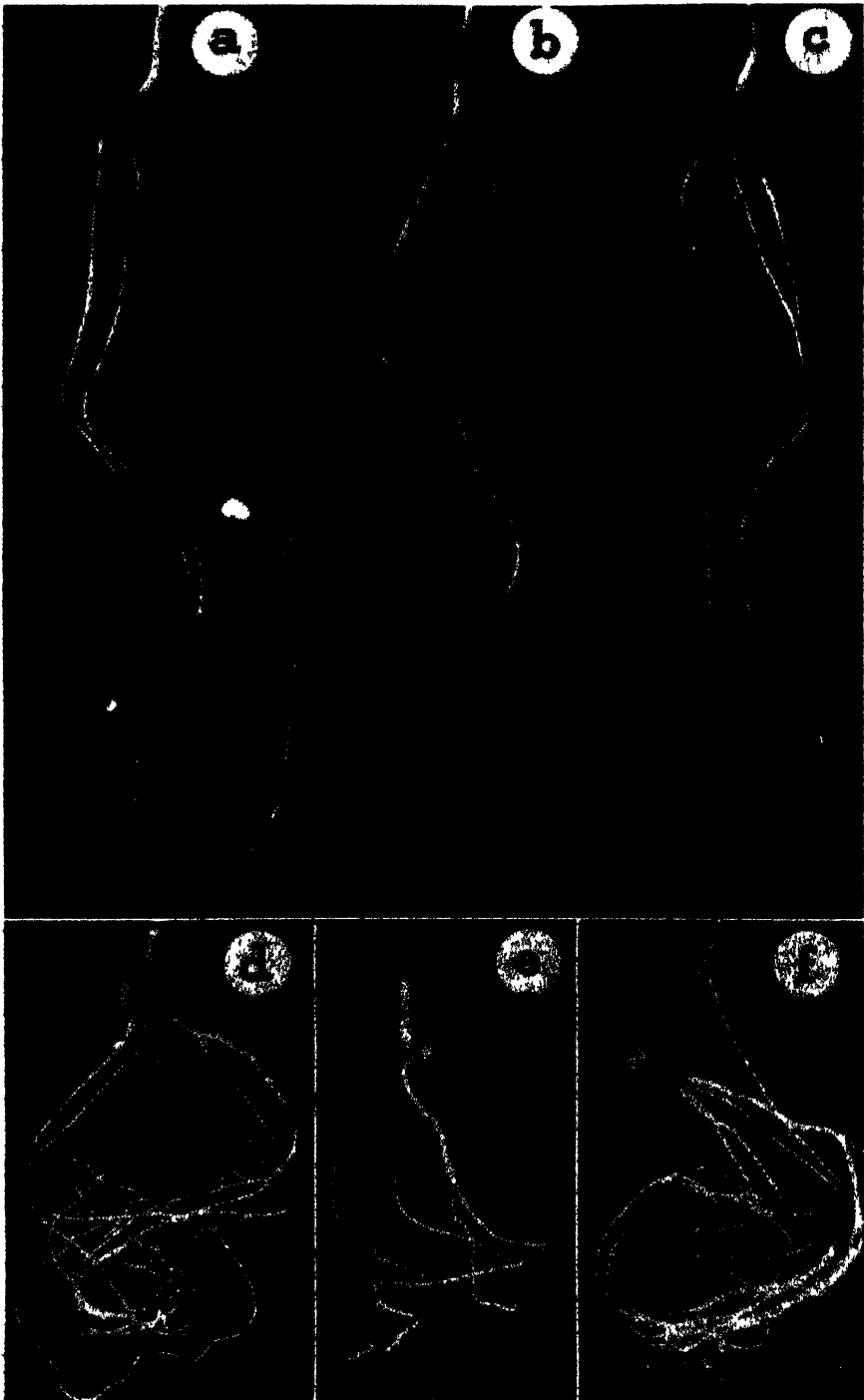
FIG. 3.   (a, b, c) Wheat roots grown 14 days, and (d, e, f) corn roots grown 18 days in soil agar: a, d, infested with culture 6 of *Streptomyces* sp.; b, e, infested with culture 7 of *S. scabies*; c, f, water control.

necrosis evident throughout the period of observation. Roots were similar in both the water control and cultures nonpathogenic to potato. However, root hair development in cultures nonparasitic to potato was variable. Certain cultures seemed to have no deleterious effect on root hairs, whereas with other cultures root hairs were very sparse or lacking. The relative abundance of root hairs was not noticeably correlated with either root or top growth.

Other species of small grains were tested with representative cultures. The following were found to be as susceptible to *Streptomyces scabies* as wheat: *Avena strigosa* Schreb.; *A. byzantina* C. Koch var. Bond; *A. sativa* L. var. Markton; *Hordeum vulgare* L. (winter and spring types); *H. nodosum* L.; *H. distichon* L. var. Spartan; *H. bulbosum* L.; *Triticum vulgare* Vill. var. Mida and an unnamed winter type; *T. durum* Desf.; *T. timopheevi* Zhuk.; and *Secale cereale* L. var. Balbo.

Corn. Plants after 12 days' growth with cultures nonparasitic to potato and in the water controls produced slender fibrous roots, whereas roots with cultures of *Streptomyces scabies* were somewhat shortened and thickened. Average root growth with *S. scabies* after 18 days was reduced (Fig. 3) to 69 per cent of either the water controls or the cultures nonparasitic to potato. Lateral roots were only slightly discolored without advanced necrosis with cultures of *S. scabies*, whereas there was no necrosis in the water controls or with cultures nonparasitic to potato. When plants were grown for 32 days, marked necrosis was evident at the root tips and the points of emergence of secondary roots, suggesting that corn was not so susceptible as wheat, pea, and soybean to *S. scabies*.

Cucurbitaceae. Of the plants tested, seedlings of cucumber and cantaloupe (*Cucumis melo* L. var. Golden Fleshed Honeydew) were the most resistant. After 18 days (Table 4), there was no appreciable difference in the type or amount of cucumber root growth in any of the cultures or in the water controls. No necrosis was evident in any culture at this time. In another test, in which plants were grown for 35 days, although roots showed little to no necrosis with *Streptomyces scabies*, they were only 72 per cent as heavy as those grown with cultures nonparasitic to potato. When the tops were accidentally injured mechanically, root necrosis promptly developed.

Roots of *Cucurbita pepo* L. var. Acorn Squash exposed to *Streptomyces scabies* were slightly necrotic in two of three trials, whereas those of *C. moschata* Duchense var. Large Cheese Pumpkin had little to no root discoloration.

Other plants. A number of small seeded vegetables and legumes, although unrelated taxonomically, had similar symptoms. Radish roots grown in the presence of *Streptomyces scabies* were necrotic at the tips and at the points of origin of laterals. Root weights (Table 4) were approximately one-half those in the water controls or with *Streptomyces* sp. nonpathogenic to potato. Where *S. scabies* was absent, plants grew well,

producing abundant lateral roots with no necrosis. As in pea, aerial parts were often stunted and hypertrophied, suggesting systemic invasion by *S. scabies*. Garden beet (Table 4) was similar to radish, except that the necrosis was extremely severe and lateral roots were almost completely inhibited.

Although onion (*Allium cepa* L. var. White Spanish) did not grow well in the soil agar medium, roots were necrotic indicating susceptibility. Tomato (*Lycopersicon esculentum* Mill. var. Pan American), although very susceptible, was slightly more tolerant than eggplant (*Solanum melongena* L. var. Black Beauty). Carrot (*Daucus carota* L. var. Imperator), parsnip (*Pastinaca sativa* L. var. Hollow Crown), and lettuce (*Lactuca sativa* L. var. New York No. 12) were susceptible.

Alfalfa (*Medicago sativa* L.), although not immune, was slightly more resistant than red clover (*Trifolium pratense* L.), alsike clover (*T. hybridum* L.), and sweet clover (*Melilotus alba* Desr.).

Seedlings grown from open pollinated seed of a number of potato varieties and progenies were tested in soil agar for resistance to root necrosis. Seedlings from scab resistant progenies, Cayuga, 116–16, 116–13, and 528–194, were apparently no more resistant than seedlings from susceptible Cobbler and Teton, although infection of cotyledons may have been a factor in the final reaction.

HISTOLOGICAL STUDIES OF PLANT ROOTS GROWN IN AGAR

Histological preparations were made to determine the relationship of the potato scab organism to plant roots developing in infested agar[5]. Blocks of agar through which wheat roots had grown were placed in killing solution ten days after the germinated seed had been planted. At this time necrosis was beginning on the root tips, but the main portions of the root appeared superficially to be unaffected (Fig. 4, A, B). Filaments of *Streptomyces scabies* were present in most of the epidermal cells and in certain of the cortical cells. Five blocks were examined from various parts of the beaker. In each case, even though the root was not visibly necrotic, streptomycetous filaments were observed in the epidermal cells. Radish roots grown eight days under similar conditions were sectioned and the organism was likewise demonstrated both in the cortical and epidermal cells. *S. scabies* culture 7 was grown on a thin agar film on a glass slide and killed, stained, and dehydrated in the usual manner. Filament diameters were similar to those in the infected cells of wheat and radish roots. No filaments were observed in wheat or radish epidermal cells with *Streptomyces* sp., culture 6, under similar conditions.

Growth of *Streptomyces scabies* was consistently stimulated in the immediate vicinity (rhizosphere) of both wheat and radish roots. Abundant filaments in the vicinity of the root were observed in temporary mounts prepared by placing blocks of agar, through which a root had grown, in

very dilute gentian violet for 1 to 2 hr. and examining without additional
treatment.   When mounts of 2 per cent agar were prepared by the paraffin
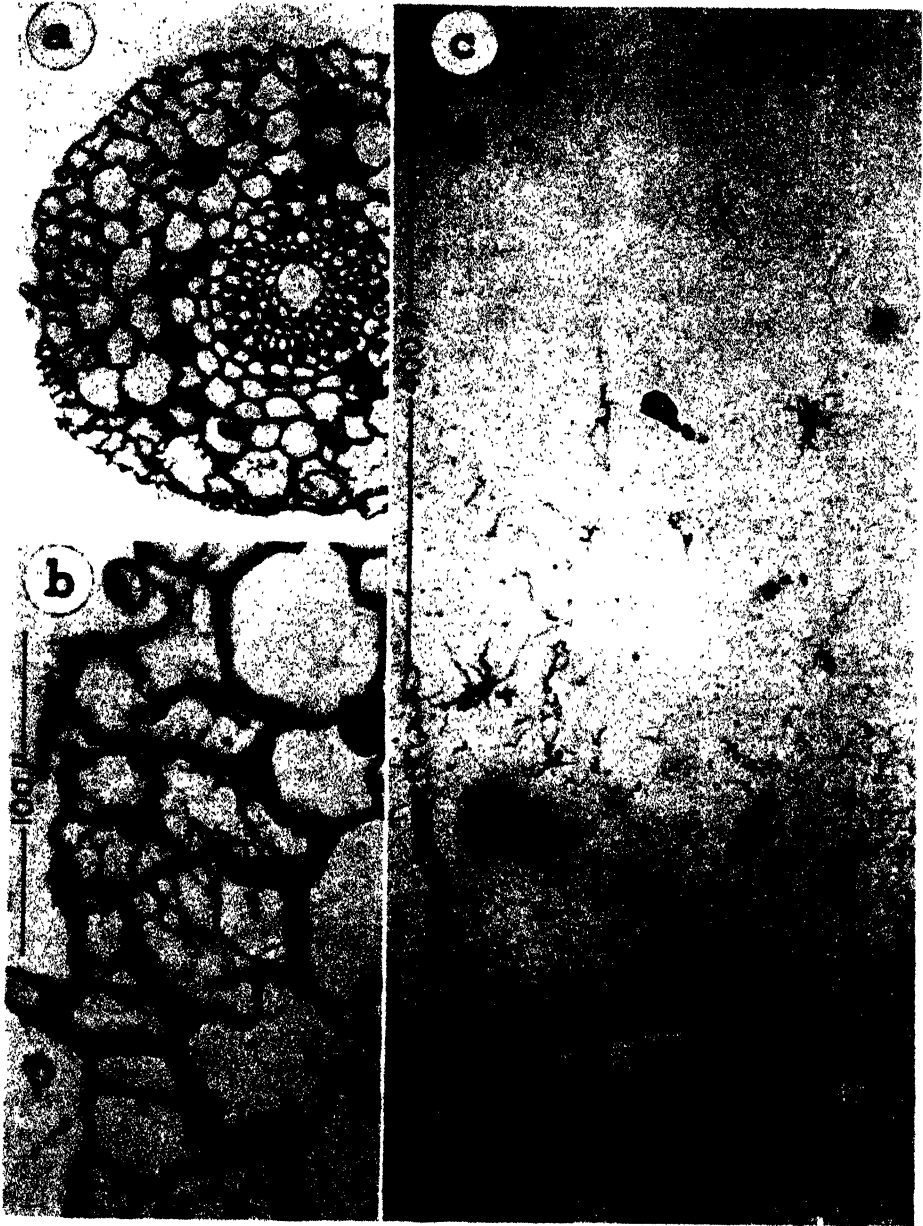
FIG. 4.   Transections of wheat root grown 10 days in soil agar infested with culture
7 of *Streptomyces scabies*: a (×120), b (×400), showing *S. scabies* filaments in the corti-
cal and epidermal cells; c (×200), with surrounding agar, showing stimulation of growth
of *S. scabies* in the rhizosphere.

method, stimulation of growth in the rhizosphere was readily demonstrated
with both wheat and radish.   Filaments of *S. scabies* were very sparse in

the medium beyond the rhizosphere. Exact measurements of the distance from a root over which stimulation of growth was apparent could not be made because of slight shrinkage of the agar during processing. In the case of wheat, the distance was measured as 0.3 mm. after processing (Fig. 4, C), and with radish it was approximately 1 mm. It is suspected that this distance might be markedly influenced by age and debility of the root. There was no evidence that nonparasitic culture 6 was stimulated to grow in the vicinity of the root of either wheat or radish.

INFLUENCE OF CULTURE FILTRATES OF STREPTOMYCES SP. ON GROWTH OF WHEAT ROOTS

Since mycelial growth of *Streptomyces scabies* was markedly stimulated in the rhizosphere of wheat and radish roots, it seemed possible that roots might have been injured by toxic substances liberated during growth of

TABLE 5.—*Growth of wheat seedlings on soil agar containing viable inoculum and filtrates of Streptomyces sp. cultures grown on potato-dextrose agar*

Material added to soil agar	Fresh root weight[a]		Secondary root development	Root tip color
	Test 1	Test 2		
	mg.	*mg.*		
Water control	139	67	extensive	white
Viable inoculum:				
culture 6[b]	147	66	extensive	white
culture 7[b]	79	30	none	orange to brown
Filtrates:				
potato-dextrose agar		50	extensive	white
20-day-old culture 6		63	extensive	white
20-day-old culture 7		56	extensive	white
50-day-old culture 6	121	56	extensive	white
50-day-old culture 7	121	56	extensive	white

[a] Records after 16 days, Test 1, 3 beakers each containing 5 plants; Test 2, 4 beakers each containing 5 plants.
[b] Culture 7, *S. scabies*; culture 6, nonpathogenic *S. sp.*

S. scabies. In order to investigate this possibility, aqueous extracts were made from 5 potato-dextrose agar mats on which *Streptomyces* sp. had been grown at 20° to 25° C. Mats were soaked for 12 to 15 hr. in 200 ml. of distilled water and the extracts sterilized by passage through a Seitz filter. Filtrates were apparently sterile since *Streptomyces* colonies failed to develop when the filtrates were streaked on potato-dextrose agar. Twenty-five ml. of the filtrate, representing the amount from 5/6 of a Petri plate culture, was placed in each beaker and 25 ml. of cool melted soil agar added and mixed. Viable inoculum consisting of a suspension of spores and aerial hyphae represented the amount obtained from approximately 1/5 of a Petri plate culture.

Growth of wheat (Table 5) in the soil agar with viable inoculum from cultures 6 and 7 was similar to that obtained in previous tests. The soil agar plus filtrates of aqueous extracts of potato-dextrose agar supported

vigorous growth of bacteria which reduced the growth of wheat slightly. In spite of the bacterial contamination encountered, wheat plants grew well, but not so well as in the water controls. Filtrates of aqueous extracts of cultures 6 and 7 supported some bacterial growth but not to the same extent as the filtrate of potato-dextrose agar alone. Approximately equivalent root weights were produced in soil agars containing filtrates of non-parasitic culture 6 or those of parasitic culture 7, which indicated that if a toxic material capable of inhibiting plant growth was produced by *Streptomyces scabies*, it was not demonstrated.

Roots in all filtrates were white at the tips, laterals were abundant, and root hairs were plainly evident. In the agar with viable inoculum of culture 7, root tips were necrotic and root hairs were either absent or very sparse. The reason why plant growth in test 1 should be almost twice as great as that in test 2 is not understood since the tests were prepared in January and March of 1947. In spite of the differences in over-all growth between the two trials, the differences between treatments within each trial were similar. In addition to the two tests reported, one preliminary trial was conducted with similar results[6].

INFLUENCE OF INOCULUM CONCENTRATION ON GROWTH OF WHEAT SEEDLINGS

During the course of the work, growth responses of wheat seedlings to *Streptomyces scabies* were constant in spite of the fact that it was occasionally necessary to use inoculum of different concentrations and ages. In general, the inoculum from 1/4 to 1/6 of a Petri plate was added to each 100-ml. beaker. To determine if such concentrations were excessively heavy, serial dilutions were prepared and beakers poured in triplicate. To each beaker 5 germinated seeds were planted. As in previous trials, root weights representing the average of 15 plants were markedly reduced by *S. scabies*. The extent of growth reduction was relatively the same when plants were grown in either a heavier than usual inoculum load of *S. scabies* or in a much lighter inoculum load than previously employed (Table 6). Although the reason is not apparent, root growth in the first trial was slightly less with the inoculum from 1/64 Petri plate than in the higher concentrations. In the second trial, root weights with cultures 5 and 9 were relatively constant throughout the range of inoculum concentrations employed.

It was suspected that variation in the time interval between pouring the agar and planting the seed might influence considerably the concentration of viable inoculum. In most cases, seed were planted in beakers within 24 hr. after pouring the agar, although in a few instances planting was delayed for 48 hr. In such cases, no variation in the final reaction was detected. In order to evaluate the effect of delayed planting, inoculum

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[6] In one other test which was at variance with those reported, some inhibition of root growth was obtained with filtrates of culure 7 and little to no inhibition with culture 6. It is suspected that bacterial growth on the filtered medium was a factor in the aberrant result.

was added and agar poured into one group of covered beakers and maintained at laboratory temperatures. Similar inoculum in Petri dishes and soil agar in flasks was held at 8° C., and after 13 days a second set of beakers was prepared. Beakers had been prepared in triplicate, and on the following day five wheat seedlings were planted to each beaker of both groups. The average fresh root weight after 13 days' growth in the delayed planting as compared to the usual planting procedure is as follows: water control, 90 and 87 mg.; culture 6 of *Streptomyces* sp., 87 and 70 mg.; culture 4 of *S. scabies*, 40 and 47 mg.; culture 5 of *S. scabies*, 24 and 44; and culture 9 of *S. scabies*, 44 and 37 mg. No well-defined trend was apparent with delay in planting, and differences were slight in the water control and in cultures 4 and 9. Root weights with nonparasitic culture 6 were somewhat lower when planting was not delayed, whereas the reverse obtained with *S. scabies* culture 5. It was concluded that the concentration of viable inoculum was influenced little, if any, by delay in planting,

TABLE 6.—*Root weight of wheat seedlings in soil agar with varying initial concentrations of inoculum*[a]

Trial number	Culture number	Fraction of Petri plate culture used as inoculum per beaker[b]									
		1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	0
		mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
1	7	56	57	53	51	47	42				87
1	6	85									
2	5		27	21	21	21	18	20	21		92
2	9		37	34	35	27	34	30	37	35	91
2	6		91								

[a] Average root weight from 15 plants grown in agar 13 days.
[b] The inoculum from 1/2 Petri plate culture represents the aerial mycelium and spores developed on 32 cm.² of agar surface. (1/256 Petri plate, 1/4 cm.²)

since with three cultures there was no appreciable effect of delayed planting, and with two cultures reverse effects were obtained.

ROOT DEVELOPMENT IN SOIL AGAR INFESTED WITH VARIOUS
STREPTOMYCETES AND BACTERIA

Soybean and radish seedlings were grown in soil agar artificially infested with 24-hr. cultures of each of the following ten members of the Eubacteriales:[7] *Serratia marcescens* Bizio, *Bacillus subtilis* Cohn emend. Prazmowski, *Bacillus mesentericus* Trevisan, *Escherichia coli* (Migula) Castellani and Chalmers. *Micrococcus pyogenes* var. *aureus* (Rosenbach) Zopf., *Sarcina subflava* Ravenel, *Aerobacter aerogenes* (Kruse) Beijerinck, *Proteus* sp., *Pseudomonas ovalis* Chester, and *Xanthomonas phaseoli* var. *sojensis* (Hedges) Starr and Burkholder. In addition, three unidentified bacterial cultures which had developed as contaminants on the soil agar in

[7] Dr. S. T. Chao of the Botany Department supplied the culture of *Xanthomonas phaseoli* var. *sojensis* and Dr. Noel H. Gross, formerly of the Department of Bacteriology, provided the other cultures tested.

previous trials were tested. Although the older portions of the roots were discolored by some of the cultures, *X. phaseoli* var. *sojensis* was the only culture which caused discoloration of the root tips of soybean and radish. The reaction was distinct from that obtained with cultures of *Streptomyces scabies* prepared at the same time, indicating that the response to *S. scabies* was specific and not obtained with any of the 13 cultures.

Ten *Streptomyces* sp. cultures isolated on soil agar from corn land that had not recently been cropped to potatoes were tested on soybean and radish[8]. None of these cultures produced a reaction typical of that already described for these plants, whereas cultures of *S. scabies* prepared under similar conditions produced a typical response.

A group of 24 *Streptomyces* sp.[9] was tested on soybean. At the time Taylor and Decker tested these cultures, 16 were nonpathogenic to potato, four were very virulent, and four were slightly virulent to potato. After approximately 10 years in culture (22), three of the four very virulent cultures were still virulent when tested on soybean, one of the four slightly virulent cultures had maintained its virulence, and none of the 16 nonpathogenic cultures was virulent on soybean. One representative culture, number 407, pathogenic to soybean and wheat, was tested on potato and found to be extremely virulent.

Ten *Streptomyces* sp. isolated from soil pox lesions on sweet potato roots were tested twice on soybean and once on wheat. Of the group, only one was typically parasitic When this culture was used to infest peat soil in which potatoes were planted, typically scabbed tubers were obtained. It is probable that this organism was *S. scabies* rather than *S. ipomoea* since it was not pathogenic to sweet potato in greenhouse tests.

Two cultures of the sweet potato soil pox organism, *Streptomyces ipomoea* (Pearson and Martin) Waksman and Henrici[10], were tested twice on wheat and slight root tip necrosis was obtained. The reaction, although not so severe as that obtained with *S. scabies*, was very similar. These cultures did not cause scab on Cobbler potatoes in greenhouse tests.

Streptomyces albus (Rossi, Doria, emend. Krainsky) Waksman and Henrici and *S. antibioticus* (Waksman and Woodruf) Waksman and Henrici[11] were tested on wheat and pea with no reaction typical of that caused by *S. scabies*.

Streptomycin produced by *Streptomyces griseus* (Krainsky) Waksman and Henrici has been reported (1) to stunt the roots of various plants such as tomato, radish, and soybean. When cultures of *S. griseus* were used on wheat, radish, beet, and soybean, roots, particularly the laterals, were stunted with no visible necrosis. The reaction was distinct from that ob-

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[8] C. H. Meredith, formerly of this department, provided these cultures

[9] The author is indebted to Carlton F. Taylor and Phares Decker for the use of these cultures, and to Wilson L. Smith, Jr., of Cornell University, who sent the transfers.

[10] One culture was isolated from soil pox infected sweet potatoes in Iowa, and a second culture was provided by Dr. W. J. Martin of the Louisiana Agricultural Experiment Station.

[11] Dr. S. A. Waksman of the New Jersey Agricultural Experiment Station made these cultures available

tained with *S. scabies* although over-all root growth was markedly reduced. Cultures of *Streptomyces* sp. were subsequently isolated from Iowa peat soils which were similar in their effect on wheat. With these soil isolates, roots were markedly stunted without macroscopically visible necrosis. Representative cultures causing this reaction on wheat were nonpathogenic to potato in greenhouse inoculation tests.

Streptomyces scabies cultures 4 and 9, isolated in January, 1944, and cultures 5 and 7, isolated in August of the same year, were transferred frequently on potato-dextrose agar. Most of this experimental work was done between January, 1946, and March, 1947. After 30 months, culture 7 lost its ability to parasitize seedling plants. The loss of pathogenicity was correlated with loss of ability to cause scab on potato in greenhouse inoculation tests. This was interpreted as further evidence that there was a positive correlation between parasitism to potato and ability to cause root necrosis and reduction in secondary roots of test plants. The pathogenicity of cultures 4, 5, and 9 remained constant over the period of observation.

DISCUSSION

The present studies show that *Streptomyces scabies* attacks a wide range of host plants under favorable conditions. The plants studied are unable to survive in artificially infested peat soil. Root infection occurs in quartz sand, and extensive root necrosis is consistently observed in soil agar. Because of the following facts the data should not be dismissed as indicating saprophytic development of *S. scabies* on weakened plants: (1) *Streptomyces* sp. nonparasitic to potato failed to attack roots of wheat, soybean, and pea; (2) the true bacteria that were tested failed to attack roots of such plants in a manner similar to that of *S. scabies;* and (3) differences in resistance to *S. scabies* were observed in the plant species tested.

Symptoms on roots grown in soil agar consisted of necrosis of root tips with a marked reduction in the number of lateral roots. Lateral roots of infected bean and pea were short stubs, whereas they generally failed to develop in infected wheat. Punctate necrotic areas on the roots of wheat were points of emergence of lateral roots in which the developing tip had been severely attacked by *Streptomyces scabies*. More resistant plants, such as certain cucurbits and corn, produced lateral roots that were shortened and noticeably thickened. Similar symptoms have been described on potato. Millard and Burr (14), using artificially infested soil, observed that the fibrous roots of potato were stunted and somewhat thickened and thickly studded with irregular dark brown outgrowths. Jones (8) demonstrated similar outgrowths to be lateral rootlets which had been partially destroyed by *S. scabies* as the lateral root emerged from the main root.

Observations on prepared sections showed that mycelium of parasitic cultures of *Streptomyces scabies* developed in the epidermal and cortical cells, becoming well established before macroscopically visible necrosis developed, whereas under similar conditions no penetration of root tissue was observed with a nonparasitic species.

In the field, streptomycetes, as well as other types of soil micro-organisms, have been reported to be much more abundant in the rhizosphere than in the soil away from the roots (9, 10, 21). In agar culture, *Streptomyces scabies* developed abundantly in the rhizosphere, whereas a nonparasitic species was not stimulated to grow in the rhizosphere. It was not determined whether *S. scabies* was stimulated to grow in the rhizosphere before actual penetration because of excretions from the healthy root, or whether *S. scabies* was stimulated to grow in the rhizosphere after penetration because of nutrients diffused from the parasitized root.

Culture filtrates of *Streptomyces scabies*, when tested under a limited set of conditions, apparently were no more toxic to wheat plants than were filtrates of a nonparasitic streptomycete. Inoculum dilution tests showed that *S. scabies* was equally effective in soil agar over a wide range of initial inoculum concentrations. Although it had been shown that growth of *S. scabies* was very poor on soil agar, it is possible that the uniform disease reaction obtained over a wide range of initial inoculum concentrations may have resulted either from growth in the low initial concentrations or from incomplete survival of inoculum in the high concentrations. These results are in contrast to those of Goss (4) in which the severity of potato scab in sterilized artificially infested soil was directly correlated with initial inoculum concentration.

No claim is made that these tests duplicate natural conditions. They do demonstrate that *Streptomyces scabies* is innately virulent to the roots of a number of plants not previously believed to be suscepts of *S. scabies*. It does not necessarily follow that the disease will be produced in the field even though it is suspected of being of more consequence on some crops than is ordinarily believed. It seems entirely possible that *S. scabies* could compete successfully with other organisms and parasitize plant roots. Beijerinck (2) reported streptomycetous filaments of *Streptothrix chromogena* Gasperini in the roots of various plants grown in the field. This organism may or may not have been *S. scabies;* his identification was based chiefly on cultural characteristics.

The presence of soil saprophytes influences considerably the parasitic ability of *Streptomyces scabies* in infested soil although published data are not in complete agreement in many details. Millard and Taylor (15) showed that in sterilized soil infested with *S. scabies*, considerable reduction in the severity of scab was obtained by introducing *S. praecox* as an antagonist. Kiessling (12) reported similar results with bacteria. Although Goss (4) was unable to obtain control of scab with *S. praecox*, he showed that scab was much more severe in sterilized, artificially infested soil than in similarly infested soil to which soil saprophytes had been introduced. Sanford (17) obtained more severe scab injury in the field by placing unsterilized, heavily infested soil around the seed piece, than by using sterilized, infested soil. KenKnight (11) experienced considerable difficulty in determining the host range of *S. scabies* because of contamina-

tion of controls by *S. scabies*. Others (22, 23), in greenhouse inoculation experiments with potato, have reported no infection or only rare infection in their noninoculated controls. Unless the host can be maintained in a culture medium relatively free from contaminants, the final result in an inoculation experiment may be unsatisfactory. This difficulty is believed to have been partially overcome, at least for small plants, by the use of the soil-agar technique.

The use of soil agar as a medium for plant root growth, in combination with streptomycetes, makes possible the testing of cultures with considerable freedom from outside contamination since the growth of most microorganisms on this medium is sparse. The medium permitted daily detailed examination of the roots. In general, plant growth was very satisfactory and roots developed extensively. Root hairs of wheat were plainly evident. The agar was easily washed from the roots permitting quantitative measurements. A group of cultures could be screened, for those parasitic to a given host, with relative ease. Satisfactory readings on potato generally require 60 days of greenhouse culture, whereas a screening on wheat and soybean can be made in 14 days, with preliminary indications available after 7 days.

The development of a dark brown ring in separated milk culture has been reported to be characteristic of *Streptomyces scabies* (22), whereas cultures which did not develop such a ring were nonpathogenic to potato. The ten cultures described in this paper were grown on milk with fair correlation between color changes in the milk and pathogenicity to potato. No culture parasitic to potato failed to give a positive reaction on skim milk. A few exceptions were observed (Table 1) in which nonpathogenic *S. lavendulae* and *Streptomyces* sp. culture 8 produced a color in milk which was indistinguishable from that of certain *S. scabies* cultures.

Although the strong possibility exists that certain isolates of *Streptomyces scabies* may be virulent to potato and avirulent to seedling test plants, it was found that a high correlation existed between pathogenicity to potato and pathogenicity to seedling plants when grown with *S. scabies* under the conditions employed. *S. ipomoea* isolates parasitic to sweet potato and nonparasitic to white potato were mildly pathogenic to wheat. Physiologic races were not demonstrated with the four *S. scabies* and six *Streptomyces* sp. cultures on such widely divergent plant hosts as soybean, pea, wheat, corn, beet, radish, and cucumber, although physiologic races as demonstrated on potato varieties and variability in *S. scabies* have been reported (19, 23).

SUMMARY

The host range of *Streptomyces scabies* on seedling plants was studied by using sterilized artificially infested peat soil, infested quartz sand, and soil agar. Scab infection of seedling plants grown in artificially infested peat soil in open pots in the greenhouse was generally unsatisfactory because of contamination. Infection of plants grown in infested peat in large

plugged test tubes was severe, resulting in infection of roots and aerial parts and premature death. *S. scabies* parasitized wheat and soybean roots developing in quartz sand, although necrosis was neither so severe nor so consistent as that on roots in soil agar. A soil-agar culture technique proved useful because cultures could be maintained relatively free from contaminants. Chronological examination of roots was possible and clean roots free of mechanical injury could be recovered.

In soil agar, four cultures of *Streptomyces scabies* caused severe necrosis, especially at the root tips, and generally precluded the development of lateral roots on soybean, pea, wheat, radish, and beet, whereas corn had slight tolerance, and cucumber considerable tolerance. Isolates of *Streptomyces* sp. that were avirulent to potatoes did not adversely affect such plants. In addition to the plants listed above, susceptible reactions to *S. scabies* were obtained with *Avena* spp., *Hordeum* spp., *Triticum* spp., rye, eggplant, tomato, carrot, parsnip, and potato seedlings. Cucurbits generally had a high degree of tolerance.

Virulent *Streptomyces scabies* cultures invaded the epidermal and cortical cells of wheat and radish roots and became intracellular. Filaments were much more abundant in the rhizosphere than in the medium a short distance away. One avirulent strain was not evident intracellularly nor was it stimulated to grow in the vicinity of the root. Aqueous extracts of *S. scabies* cultures were no more toxic to wheat roots than extracts of cultures nonparasitic to potato. *S. scabies* was equally parasitic to wheat roots over a wide range of initial inoculum concentrations, and the growth response was constant where planting was delayed by as much as eight days.

Although a total of 60 *Streptomyces* sp. cultures were tested on wheat, with the exception of *S. ipomoea*, only those known to cause potato scab parasitized wheat seedlings. *S. ipomoea* caused necrosis of wheat roots in a manner similar to that of *S. scabies*, although the reaction was much less severe. Root necrosis was not obtained when cultures of 13 members of the Eubacteriales were tested on seedlings growing in agar. In the few instances where root discoloration developed, the reaction was distinct from that caused by *S. scabies*.

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A LATE-BREAKING VIRUS DISEASE OF POTATOES[1]

J. A. MILBRATH AND W. H. ENGLISH[2]

In 1946 an unusual disease of potatoes became prominent in many potato fields in Oregon, most fields of Netted Gems, Burbanks, and White Rose having from a trace to 3 per cent of the disease. After all seed lots with more than 1 per cent infection were rejected for certification, prevalence was lower in 1947 plantings. Seed obtained directly from Montana, Colorado, and Idaho produced plants with tuber-perpetuated symptoms, an indication that the disease is rather widespread.

Vine symptoms suggested Rhizoctonia and leafroll complexes, but closer observations indicated that neither disease was involved. Subsequent studies demonstrated the disease to be of virus nature, but possessing certain features which indicated that it was different from viroses heretofore associated with potatoes in Oregon. The extensive distribution of the disease in Oregon and its unusual nature suggested this preliminary paper.

SYMPTOMS

This disease was first noted in the Netted Gem variety, in plants which appeared to be showing the effect of current season infection. Most of the affected plants were of normal size and frequently only one branch or even one lateral was affected. However, when studied in a field planted in tuber units it became apparent that the disease was tuber-perpetuated.

When the symptoms are first expressed, the tips of the laterals begin to roll much as they do when the roots are injured by Rhizoctonia or some other factor, or when the plants are infected with current season leafroll. The rolling closely resembles that illustrated by Leach and Bishop (7) for Purple-top wilt. Subsequent symptom development is from the tip downward until the entire plant becomes involved. In a few days the plants become stiff and erect, with olive green to chlorotic foliage. They often have a purplish cast from heavy pigmentation of the leaf veins, the stems, and the small rolled leaflets in the branch terminals. In the more advanced stages some plants wilt and die, apparently from root starvation and subsequent rot. Most plants in the advanced stages of the disease produce aerial tubers, often in nearly every leaf axil.

The disease was studied in fields which had been planted in tuber units, and considerable variation was noted in the degree of severity of symptoms. Occasionally the entire unit was dwarfed and rolled much in the same manner as for leafroll, but for the most part the units contained

plants which had developed to nearly normal size before expressing symptoms. In some units all plants were affected in a uniform manner, but in others normal plants, plants with mild symptoms, and plants with acute symptoms could be found, all from the same tuber.

Occasionally a seed piece may not produce a plant large enough to reach the surface of the soil. Short stolons with small terminal tubers are the only structures produced (Fig. 1, C). The seed pieces from diseased

FIG. 1. Late breaking virus disease of Netted Gem potato. A. Right, weak plant from infected tuber grown in greenhouse; left, a normal plant. B. Field plant with early acute symptoms. C. Dormant seed piece with small secondary tubers. D. Infected Netted Gem tuber producing small weak sprouts.

tubers are resistant to breakdown and may remain firm in the soil for several months. Some seed pieces produce knots along the cut vascular ring, while others force out a well defined root system from the cut vascular ring.

In general, the appearance and behavior of the disease in the White Rose and Burbank varieties is similar to that in Netted Gems. However, the late-breaking or late-developing symptoms are not so striking on White

Rose as on Netted Gems, and the disease is extremely difficult to distinguish by foliage symptoms from that caused by Rhizoctonia or other similar root injuries. Its expression usually is limited to an apical leafroll followed by aerial tuber development in nearly every leaf axil If a White Rose plant shows the foliage symptoms described above and there

FIG. 2. Tuber perpetuated late breaking virus disease of White Rose potato A. Aerial tubers. B. Downcurving axillary branches. C. Plant terminal 60 days after inoculation by grafting. D. Aerial stolons.

are no evident root injuries and the tubers are smooth and small, the disease will be reproduced by many of the tubers. Some infected White Rose plants develop the early and acute symptoms that resemble tuber-perpetuated leafroll. The plants are dwarfed and chlorotic as for leaf-

roll but they do not express the rolling, cupping, and bronzing of the lower leaves.

Tubers from acutely diseased plants are reduced in size but have no internal discoloration or other evidence of disease. Most of the tubers are firm and show no indication of wilting or flabbiness unless the plant suddenly wilts and dies from what appears to be a secondary invasion of the weakened or starved roots by Fusarium and other soil organisms. Many of the larger tubers from infected hills produce hair-sprouts, either from all of the eyes or from a portion of the tuber (Fig. 1, D).

Tubers taken from infected hills of Netted Gems and planted in the greenhouse produced plants varying greatly in symptom expression.

TABLE 1.—*Location of the virus in the tuber*

Tuber number	Apical left	Basal left	Apical right	Basal right
1–30	–	–	–	–
31	–	+	–	+
32	+	+	+	+
33	+	+	–	+
34	–	+	+	+
35	–	+	–	–
36	+	–	+	–
37	+	–	+	–
38	+	–	+	–
39	–	+	–	+
40	+	+	+	+
41	+	+	+	+
42	–	+	–	+
43	–	–	–	+
44	–	+	+	+
45	+	–	+	–
46	–	+	+	–
47	–	+	–	+
48	+	–	+	–
49	+	+	+	+
50	–	+	+	+

Often the plants appear normal or vary only slightly from normal. There is a decided tendency for delayed emergence, and for weak (Fig. 1, A) and hair-sprout plants. Occasionally a plant will develop in a normal manner until 17 in. or more tall, then terminal growth will cease and purple aerial tubers or short, vase-shaped shoots will develop in the axils of the leaves as illustrated for White Rose in figure 2. These plants soon become chlorotic and when they reach a certain stage of development many of them wilt and die within a few days.

Symptoms in greenhouse-grown White Rose are much more clear-cut than on Netted Gems. In addition to dormant seed pieces, hair sprouts, and weak plants, nearly every infected plant may produce profuse axillary aerial tubers, abnormal, downward-curving leafy branches, or aerial stolons with or without tubers attached (Fig. 2, A, B, D).

In potato fields, several plants were noted with two stalks from the

same seed piece: one stalk had acute symptoms and tubers were small; the other appeared to be healthy, with normal tubers.   In an experiment, one tuber from a diseased stalk and another from a normal stalk, taken from each of ten different hills, were segregated and planted in the greenhouse. All of the tubers from the diseased stalks produced diseased plants; those from the normal stalks produced nine normal plants and one diseased plant.

Fifty tubers weighing from 6 to 8 ounces were selected from hills having mild symptoms of the disease.   Each tuber was cut once longitudinally and once crosswise, and the four seed pieces were placed in field plot rows so that the pieces were oriented as to their position in the tuber.   Table 1 shows the results of these studies.

Thirty of the tubers produced apparently normal plants from all four seed pieces.   The other 20 tubers produced from 1 to 4 diseased plants. The virus was not always present throughout the tuber, but was often localized in some one area.

INOCULATIONS

Netted Gems and White Rose have been inoculated by grafting, using infected plants of both varieties as sources of the disease.   Approximately 60 days after grafting a portion of the infected plant onto small healthy plants, an apical leafroll developed and axillary tubers appeared in the upper portions of the plants (Fig. 2, C).   Inoculated plants became decidedly yellowed and the leaves rolled upward.   Plants grown from tubers produced by the inoculated Netted Gem plants, whether or not current season symptoms were evident, invariably developed typical symptoms. The progeny from the inoculated White Rose plants, however, failed to produce symptoms, again regardless of current season expression of the disease.   Check plants originating from the same tubers as the inoculated plants remained normal throughout the experiment.

Although the disease is of the yellows type which is usually not transmitted by juice inoculations, five plants were inoculated using carborundum powder and the juice taken from plants having early but well-defined symptoms of the disease.   The plants were grown to maturity but none developed any type of abnormal growth that differed from the uninoculated check plants.   Table 2 summarizes the results of the inoculation studies.

DISCUSSION

The name "late-breaking virus disease" has been proposed and is being used locally as the common name of the disorder.   The name is derived from the fact that the disease, even when tuber-perpetuated, often does not express itself until late in the season at which time its development is very rapid.

For a comparison with other similar virus diseases or disease complexes

described for potatoes, the following points pert.nent to the late-breaking virosis should be noted: (1) repeated demonstrations of tuber perpetuation for two or more generations; (2) no internal necrosis of stems or tubers; (3) tubers usually borne on short stolons, small but not in chains or excessive in number; (4) profuse production of aerial tubers in the field and aerial tubers and aerial stolons in the greenhouse; (5) uneven distribution of the virus in the tuber; (6) hair sprouts from large tubers but no tendency for multiple sprouting or branching to produce witch's-broom effect; (7) the production of dwarf plants that show acute symptoms early (Fig. 1, B), or apparently normal plants that develop symptoms late in the season, often from the same tuber.

This disease has many features which suggest that it belongs to a group of viroses which for the most part have not been sufficiently investigated to clearly demonstrate their relationships. Its erratic behavior and dif-

TABLE 2.—*Inoculation studies demonstrating the transmission of late breaking virus disease to White Rose and Netted Gem potatoes*

Variety	Source of inoculum	Type of inoculation	Number of plants	No. of transmissions	
				Current season	Tuber perpetuated
White Rose	White Rose	graft	9	7	0
White Rose	Gems	graft	6	5	0
Gems	Gems	graft	11	4	11
Gems	White Rose	graft	9	7	9
White Rose	Checks	none	3	0	0
Gems	Checks	none	6	0	0
Gems	White Rose	juice	5	0	0
Gems	Checks	juice	3	0	0

ferent symptom expression at times suggest relationship to Purple-top wilt (7), Yellow Top (3, 4, 9), Witch's Broom (6), Apical Leafroll (1, 8). Haywire (5), and Bunch top (2). However, the points listed above appear to differentiate this "late-breaking" virus disease from these previously described and somewhat similar disorders.

This disease does not have the constant, clear-cut symptom expression of many of the other potato virus maladies such as mosaic and leafroll. This will make it difficult for growers to eliminate it from their seed stocks. The delayed symptom development and the uneven occurrence within a tuber makes the disease hard to detect in eye index and winter test plots. The tendency for the majority of infected Netted Gem tubers to develop only weak or leggy plants with no definite foliage symptoms makes greenhouse detection and study difficult. For the same reason trouble is being experienced in vector studies that now are in progress. This virus disease presents a number of intriguing research problems that remain to be solved.

SUMMARY

An unusual virus disease has been found in Oregon which possesses certain characteristics that differentiate it from other viroses associated with potatoes. It has been called "late-breaking virus disease" because the symptoms on plants produced from infected tubers usually develop late in the season. The disease has been tuber-perpetuated for at least two generations and has been transmitted to healthy plants by grafting. The disease is very erratic in behavior and symptom expression, a fact that will make it difficult to detect and eradicate from seed stocks.

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A CRATER SPOT OF CELERY PETIOLES CAUSED BY RHIZOCTONIA SOLANI

BYRON R. HOUSTON AND J. B. KENDRICK [1]

For many years a petiole crater spot of celery has been present on plants in the fields in the Sacramento-San Joaquin Delta of California. The severity of the disease varies to some extent with the season and usually increases with continuous celery culture. Losses result when the outer petioles become infected and must be removed before marketing. Often the infections occur to such an extent that excessive trimming makes it impossible to market the small or improperly shaped plants. Both the Pascal and Self Blanching types of celery are susceptible. The investigations herein reported were undertaken to determine the primary cause of this disease.

The first evidence of the disease is seen on the outer petioles in contact with the soil. Infections occur on either the dorsal or ventral surface of the petiole. The lesions first appear as small tan spots (Fig. 1, B, C) which may show a slight margin of watersoaked tissue if in very moist soil. As the spots enlarge they become darker brown and sunken in the center as the tissues collapse and dry (Fig. 1). On the dorsal surface the lesions are often elongated an inch or more in the direction of the long axis of the petioles. The parenchyma tissue between the veins collapses leaving the veins as ridges through the spots (Fig. 1, B). Infections several inches above the crown occur after ridging of the soil against the plant a short time prior to harvest to produce a partial blanching. Infections occurring on rapidly elongating petioles result in a scarring of the surface tissue and often a distortion (Fig. 1, F).

Isolations made in the fall of 1947 from field specimens of the disease yielded a number of fungi and bacteria. The most common fungi isolated were *Rhizoctonia solani* Kuhn and what appeared to be *Fusarium solani* (Mart.) App. et Wr. emend. Snyder and Hansen. *Erwinia carotovora* was isolated many times, as were a number of other bacteria. Ark (1) reported a crater rot of celery from the Delta region as being caused by *E. carotovora*. His published photographs of the disease show it to be very similar to the disease reported here. Bardin and Fong (2) reported a Phoma root and crown rot of celery from California which produces dark-brown to black lesions around the base of the petioles and on the roots, but does not produce sunken spots with a sharply limited margin. This was apparently the same disease as that earlier described by Bennett (3). In 1907 Van Hook (5) reported a celery root rot from Ohio with which *Rhizoctonia* was

associated, but he was unable to reproduce the disease in inoculations with this organism.

The pathogenicity of the various fungi and bacteria isolated from the
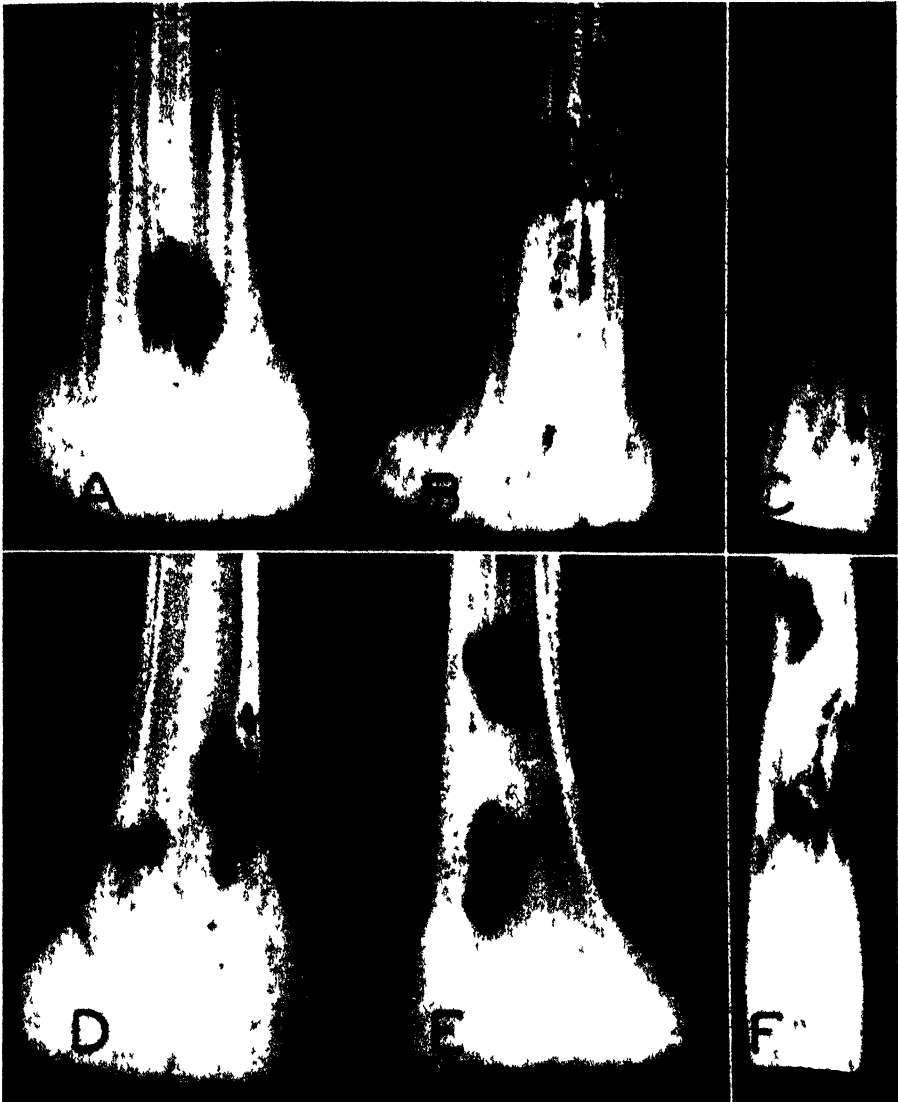
FIG. 1. Field specimens of celery petiole rot caused by *Rhizoctonia solani*. A, B, and C. Dorsal surface of petiole. D, E, and F. Ventral surface of petiole. A, D, and E. Typical sunken lesions following the drying of the affected tissue. B. Elongate lesion on dorsal surface showing sunken tissue between veins. C. Young infections somewhat watersoaked prior to discoloration. F. Multiple infection of young petiole resulting in distortion.

field specimens was tested in the greenhouse in the spring of 1948. Celery plants of the Pascal type approximately 4 months of age growing in deep flats of sterile peat soil were inoculated by pouring macerated agar cultures

of the organisms around the crown of the plant and between the petioles. The soil was then pulled up around the base of the petioles and kept moist for 3 weeks. Ten plants were inoculated with each organism. Preliminary observations at the end of 5 days showed that none of the organisms had penetrated the tissues of the celery petioles, with the exception of *Rhizoctonia solani* which had produced small tan lesions 4 to 8 mm. in diameter on both the dorsal and ventral sides of the petioles. At the end of the 3-week period the plants were dug and examined and it was found that no organisms other than *R. solani* had infected the petioles This

FIG 2. Lesions on celery petioles following greenhouse inoculations with *Rhizoctonia solani*.

fungus had produced multiple infections with resulting lesions several centimeters in width (Fig. 2). Isolations from these infections were approximately 90 per cent *R. solani* which appeared identical to those isolates used for inoculation.

It has been found difficult to consistently isolate *Rhizoctonia solani* either from field specimens or greenhouse inoculations because of secondary bacterial contaminants of nonpathogenic types which tend to suppress the growth of the *Rhizoctonia*. Good results were obtained by isolating from the younger infections with the use of acidified potato-dextrose agar.

Inoculations with isolates of *Rhizoctonia solani* obtained from sugar beet

and potato stem lesions showed these isolates to be very pathogenic on celery. All of the isolates obtained from natural celery infections as well as those from other hosts were found to be of the type designated as type A by Houston (4).

The results of soil inoculations are given in table 1. Four isolates of *Rhizoctonia solani* and one of *Erwinia carotovora* from celery were mixed into flats of soil prior to planting with healthy celery plants about 3 months old. In these trials *R. solani* caused the death of about 17 per cent of the transplants by complete invasion of the crown tissues subsequent to the crater spot. The remainder of the plants were all severely damaged by typical crater-spot petiole infections. Infection by *E. carotovora* occurred at a few places on the outer petioles apparently at points of injury.

Further trials with *Erwinia carotovora* on growing plants and on excised petioles in moist chambers showed that this organism was capable of entering the tissue only when a wound was present. Puncturing with a

TABLE 1.—*Crater spot infection following the infestation of sterile soil with cultures of Rhizoctonia solani and Erwinia carotovora isolated from celery prior to planting with healthy celery plants*

Isolate number	Number of plants:		Average severity of crater spot infection[a]
	Planted	Surviving	
R. solani 1	40	32	2.6
R. solani 2	40	39	2.9
R. solani 4	40	35	3.0
R. solani 7	40	27	2.6
E. carotovora + 1	40	40	0.2
Sterile soil	40	40	0.0

[a] Rating based upon number of crater spot lesions on petioles and number of petioles infected: 0 = healthy and 4 = dead.

needle at the time of inoculation resulted in infection and the formation of a typical crater of watersoaked tissue followed by collapse of the internal parenchyma of the petiole several centimeters in each direction from the point of inoculation. No typical crater spot was produced. When a suspension of *E. carotovora* was poured around the crown of plants which had 5 days previously been inoculated with *Rhizoctonia solani* the bacteria became established in the areas injured by the fungus on 12 of the 50 plants inoculated. On 7 of the 12 plants the bacteria completely invaded the crown tissues resulting in the death of the plant. A soft rot occurred on some of the petioles of the other 5 plants.

The evidence presented indicates that *Rhizoctonia solani* is the primary cause of crater spot and that *Erwinia carotovora* will produce a soft-rot lesion by invasion through mechanical wounds or through lesions produced by *R. solani*.

SUMMARY

A petiole crater-spot of celery was found rather widely distributed in the Delta region of California. The disease was characterized by tan to

brown, ovoid, sharply sunken lesions on the dorsal or ventral surface of the petiole often somewhat more elongated on the dorsal surface.

Although a number of fungi and bacteria were isolated from the diseased plants, the only organism which proved to be pathogenic was *Rhizocton'a solani*. Isolates of this fungus from sugar beet and potato stem lesions also were pathogenic on celery.

Erwinia carotovora produced petiole rot when inoculated through mechanical wounds on celery petioles or when inoculated subsequent to *R. solani* infections. In the latter case complete invasion of the crown tissue may occur.

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DECAY OF CERTAIN NORTHERN HARDWOODS BY FOMES IGNIARIUS, PORIA OBLIQUA, AND POLYPORUS GLOMERATUS

RAY R. HIRT

One of the most important heartrot fungi of nonconiferous trees, *Fomes igniarius* (L. ex Fries) Gill., is common in hardwood stands of New York State. At the Charles Lathrop Pack Demonstration Forest at Warrensburg, New York, it occurs frequently on hardwood trees of various species and is especially common on aspen. The sporophores of *Fomes igniarius* on aspen differ somewhat in appearance from those produced by the fungus on trees of other species. In culture, the isolates from aspen also differ in certain respects from isolates of the fungus taken from other hardwood hosts (2, 5). It has been stated that the aspen form grows only on aspen (5). If *Fomes igniarius* from aspen should be able to grow on other hardwood species, then a heavily infected aspen stand in close proximity to other hardwoods could be a serious source of inoculum and of concern in the management of hardwood stands.

In 1938 a project was begun at the Pack Forest to discover whether isolates of *Fomes igniarius* from trees of various hardwood species could grow in hardwood hosts of species other than those from which the isolates were obtained. Since sterile conks on birch and beech at that time were thought to be formed by *Fomes igniarius*, they were included in the study. It was known that sterile conks on beech were not always associated with that fungus (4)

THE ISOLATES

Isolates were taken in the spring of 1938 from the following trees bearing fertile sporophores of *Fomes igniarius*[1]: aspen (*Populus tremuloides* Michx.); beech (*Fagus grandifolia* Ehrh.); elm (*Ulmus americana* L.); and ironwood (*Ostrya virginiana* [Mill.] K. Koch). Isolates were secured also from sterile rimose conks on yellow birch (*Betula lutea* Michx.) and beech. The isolated mycelia were grown in culture and their growth characteristics compared with those recorded by Campbell (2) and by Verrall (6) for *Fomes igniarius*.

The isolates from fertile sporophores of *Fomes igniarius* and the associated decayed wood grew, with minor variations, in the characteristic manner in culture. The isolate from the fertile sporophore on beech grew more rapidly than any of the other isolates on malt extract agar. However, in all other respects it was typical of *F. igniarius* cultures described by Campbell. The isolate from aspen was similar also to Campbell's descrip-

tion of *F. igniarius* in culture except that it had a pronounced wintergreen odor.

The fungi isolated from the sterile conks were different from *Fomes igniarius* in their cultural characteristics. The isolate from the sterile conk on beech proved to be *Polyporus glomeratus* Peck. The fungus cultured from the sterile conk on birch was identified by Ross Davidson of the Division of Forest Pathology, Beltsville, Maryland, in May, 1938, as belonging to the *Poria obliqua* complex. Fertile sporophores produced on dead trunks of yellow birch bearing sterile conks in the stand where the isolate was secured were identified by Dr. J. L. Lowe of The New York State College of Forestry as *P. obliqua* (Pers.) Bres.

INOCULATION OF HARDWOODS

Healthy, vigorously growing forest trees of trembling aspen, beech, yellow birch, sugar maple (*Acer saccharum* Marsh), and ironwood growing

TABLE 1.—*The hosts from which the three heartrot fungi were isolated and the hosts in which they became established following artificial inoculation*

Isolate and source	Success[a] of inoculation				
	Aspen	Ironwood	Maple	Beech	Birch
Fomes igniarius					
Aspen	5/5	1/1	0/1	1/1	1/1
Ironwood	1/1	5/5	1/1	1/1	1/1
Elm	2/2	1/1	3/5	1/1	1/1
Beech	1/1	1/1	1/1	4/4	1/1
Poria obliqua					
Birch	1/1	0/1	1/1	1/1	3/3
Polyporus glomeratus					
Beech				2/2	1/1

[a] The denominator indicates the number of inoculations; the numerator, the number of successful inoculations where the fungus became established and caused decay.

in the Pack Forest at Warrensburg were inoculated with the isolates of *Fomes igniarius* and *Poria obliqua* in July, 1938. Beech and yellow birch only were inoculated with *Polyporus glomeratus* in July, 1939.

Inoculation of the trees was accomplished by drilling holes into the heartwood of the trees and inserting the agar substratum covered with vigorously growing mycelium. Care was taken to avoid contamination of the inocula during the procedure. Each hole was plugged with a sterilized birch dowel, which was immediately brushed with white lead paint. The trees were observed several times each year until they were felled in October, 1948, and split longitudinally, and the decay was studied.

RESULTS

Regardless of the host from which cultures of *Fomes igniarius* had been secured, except for the aspen isolate on sugar maple, each isolate became established and caused decay within vigorously growing trees of aspen,

beech, yellow birch, ironwood, and sugar maple growing under forest conditions (Tables 1 and 2). *Poria obliqua* from yellow birch became established in aspen, beech, birch, and maple. *Polyporus glomeratus* isolated from beech caused decay in beech and birch, the only two species of trees in which it was introduced.

Decay

The amount of decay resulting from inoculation with a single isolate differed greatly between trees of different species (Table 2). The amount of decay that developed within trees of the same species also varied with the different isolates.

These variations in decay between the individual trees over the 10-year

TABLE 2.—*The hosts from which the heartrot fungi were isolated and the vertical extent of heartrot developed in the inoculated trees after 10 years*

Isolate and source	Amount of vertical heartrot in				
	Aspen	Ironwood	Maple	Beech	Birch
	Ft.	Ft.	Ft.	Ft.	Ft.
Fomes igniarius					
Aspen	7.09	5.33	0.00[b]	1.91	11.00
Ironwood	3.83	8.06	13.00	[c]	15.25
Elm	23.00	1.33	18.00	2.50	1.08
Beech	3.00[a]	11.33	6.50	1.41	10.50
Poria obliqua					
Birch	25.00	0.00[b]	0.25	0.66	23.66
Polyporus glomeratus					
Beech	.			3.50[d]	14.00[d]

[a] Extent of decay at the end of 5 years when tree was accidentally destroyed during cutting operation.
[b] Inoculation failed to take.
[c] Although decay had developed, an extensive bark canker had also formed permitting other decay fungi to enter, making it impossible with certainty to trace decay by *Fomes igniarius*.
[d] Amount of decay in 9 years.

period are to be expected. Individual trees within a species are known to vary measurably in durability (7).

Aspen and yellow birch developed rather extensive decay from both *Fomes igniarius* and *Poria obliqua*. In aspen the decay caused by *P. obliqua* was typical of the decay associated with that fungus in yellow birch.

Fomes igniarius averaged less decay in beech than in any of the other host species (Table 2), although it has been stated that beech in the Adirondacks is severely affected by this fungus (1). It is possible for a fungus to become established readily on exposed dead wood of wounds and still develop slowly in the heartwood of live trees. Much of the infected beech in the Adirondacks is mature or overmature and it is possible for *F. igniarius* to have been present for many years in beech trees.

The failure of the *Fomes igniarius* from aspen to cause decay in the single maple tree does not mean that it cannot become established in sugar

maple. The fungus was dead in the inoculation chamber when the tree was cut but there was no indication why it had died. The surprising fact is that *F. igniarius* became established so readily in the various live hosts following artificial methods of inoculating them.

Sporophore Production

Two fruit bodies of *Fomes igniarius* were produced on the inoculated hosts.

On ironwood inoculated with *Fomes igniarius* from beech, a fertile sporophore became visible in June, 1944, 6 years after the inoculum was planted in the host (Fig. 1, A). The sporophore continued to develop until the tree was cut in 1948. In appearance it resembled the normal sporophore of *F. igniarius*, and the decay associated with it was typical of that produced by this fungus in other hardwoods (Fig. 1, B).

A sporophore began to form by July, 1948, on aspen inoculated with an isolate from aspen (Fig. 1, D). Basidiospores were present when the tree was cut the following October.

DISCUSSION

The fact that *Fomes igniarius* taken from trees of several species of northern hardwoods was established by artificial means in other hardwood species and caused decay, suggests that under natural conditions this exchange of hosts may occur. If this is true, infected stands of aspen in close proximity to other valuable hardwood species may serve as an important source of inoculum and consequent decay. This is especially important because *F. igniarius* may produce fertile sporophores on aspen when the trees are relatively young. During cutting operations in hardwood stands, the removal of all trees infected with *F. igniarius* is a good safety measure.

Poria obliqua is relatively common on yellow birch in the Adirondacks. In this study it caused very limited decay in beech and sugar maple, but very extensive decay in aspen and yellow birch within 10 years. The author is not aware that this fungus has been reported on aspen following natural inoculation. Perhaps the species of hardwoods that are subject to decay by this fungus in America are more extensive than present records indicate.

Polyporus glomeratus isolated from a sterile conk on beech caused decay in beech and birch. Somewhat less than 4 years after the beech tree with a sterile conk was cut and left on the forest floor, fertile sporophores of *P. glomeratus* were produced in great abundance. *Poria obliqua* on birch also produces fertile sporophores several years after an infected tree dies (3). Establishment of sterile conk fungi such as *P. glomeratus* and *P. obliqua* by means of basidiospores is dependent upon the production of fertile sporophores. Therefore during cutting operations it is good practice to remove birch and beech trees bearing sterile conks, and dispose of the infected logs in such a way that the fungi cannot produce the fertile sporo-

phores on the dead wood   This should result in reducing heartrot caused
by these fungi.
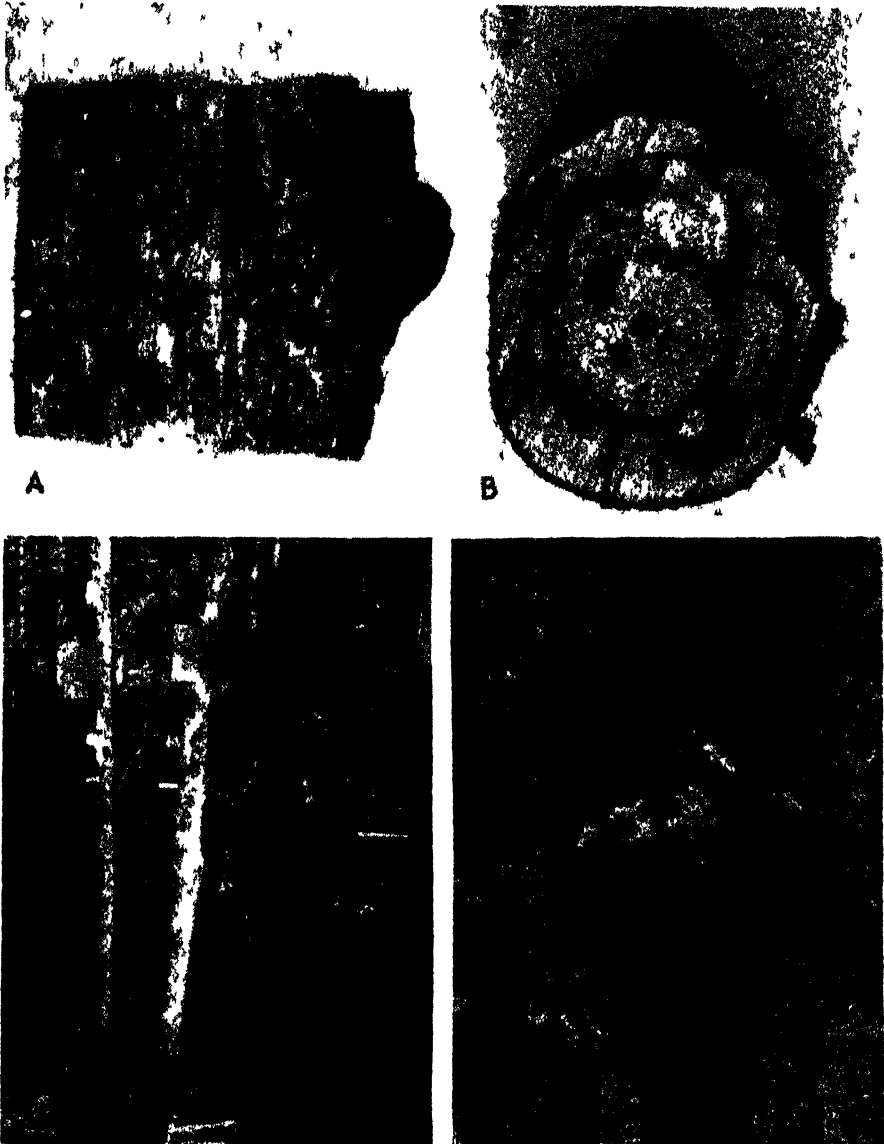
FIG 1   A Sporophore on ironwood produced by *Fomes igniarius* isolate taken from
beech.   B. Cross section of ironwood just above the sporophore showing typical white
heartrot.   C. Ironwood and sugar maple after inoculation with *F igniarius* isolates.
D. Young sporophore on aspen produced by *F igniarius* isolate from aspen   (Photo
graphs by J. L Lowe)

SUMMARY

Fomes igniarius was isolated from trembling aspen, beech, yellow birch,
elm, and ironwood growing in the Pack Demonstration Forest at Warrens-

burg, New York. Forest trees of aspen, beech, yellow birch, ironwood, and sugar maple were inoculated with each of the isolates. Regardless of the host-source of the inoculum, the isolates became established in all of the represented species except one. Appreciable heartrot developed in all the host trees.

Poria obliqua isolated from yellow birch became established and caused extensive decay in aspen and yellow birch, but relatively little decay in beech and sugar maple.

Polyporus glomeratus was isolated from a sterile conk on beech and inoculated into healthy beech and yellow birch. Within both hosts it caused decay.

Sporophores of two isolates of *Fomes igniarius* were produced on two trees: on ironwood in 6 years by a beech tree isolate; on aspen in 10 years by an aspen isolate.

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PIT CANKER OF ELM[1]

NESTOR E. CAROSELLI AND C. M. TUCKER[2]

INTRODUCTION

The pit canker disease of elm has been known to arborists for over forty years but its cause has heretofore been undetermined. This abnormal pitted condition of elms has been associated with insects by Stone (4) in whose publication there is a photograph of a pitted trunk. He reported that pitting was often noted on trees growing "under uncongenial conditions."

Other authors have also found that in the majority of cases trees having the disease were growing under poor conditions. Felt and Rankin (2) referred to this disease of undetermined origin as pit canker and reported it to be frequently found affecting elms in New England. A similar canker of elm was described by Fox (3) who contended that the injury occurred as a result of horses chewing the bark of trees. Cankers have been noted occurring on old trees which had rings to which horses were tied. However, cankers on these old trees were observed also at heights of 15 to 20 feet, well out of reach of these animals. This disease is often referred to by arborists as deep pitted canker, deep canker, elm trunk canker (1), and ring canker.

DISTRIBUTION AND ECONOMIC IMPORTANCE

The American elm (*Ulmus americana* L.) is the most susceptible species but the disease has also been found on slippery elm (*U. fulva* Michx.). Samples sent to the Bartlett Tree Research Laboratories for diagnosis indicate that the disease is present in Massachusetts, Connecticut, New York, and Pennsylvania. Dodge (1) reported the disease on trees growing in Montreal, Canada, and in the Middle Atlantic States.

Although the disease has no sudden and drastic effects on the tree, its presence often results in decline. Numerous cankers on the trunk mar the beauty of the tree (Fig. 1, A.). Severely infected trees become unsightly and their removal is necessary. As a rule, the disease is present in a few trees in scattered localities; in certain areas, however, over 60 per cent of the elms are infected. The prevalence of the disease is illustrated by the report of Dodge (1) that within ten years 10 per cent of the elms in a small Massachusetts town became infected.

SYMPTOMATOLOGY

External symptoms of pit canker are the formation of concentrically marked cankers on the trunk and sometimes on the scaffold branches (Fig.
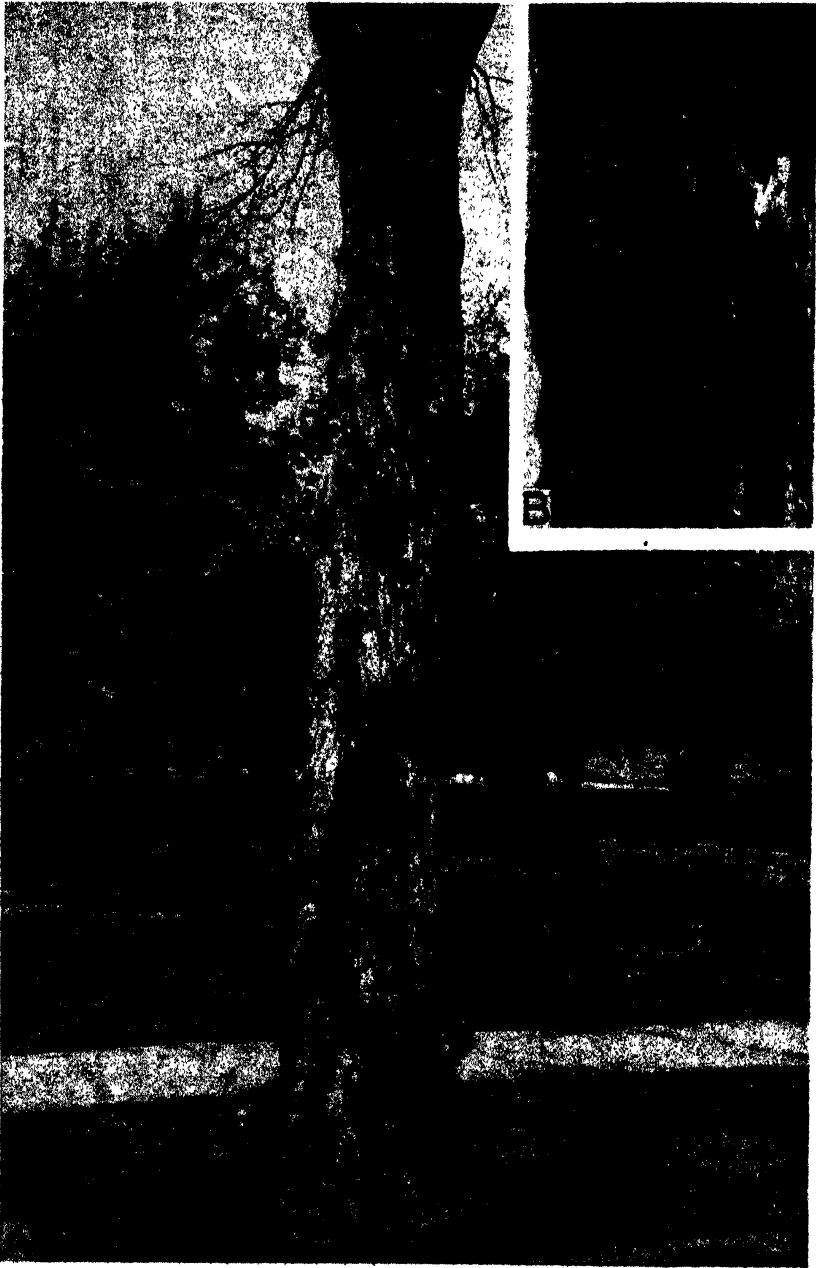
FIG. 1. A. Elm tree showing irregular trunk typical of pit canker disease. B.
Close-up of trunk with characteristic pit cankers.

2, B.).   The characteristic zoning in the cankers probably represents the
yearly progress of the causal organism.   After injury is caused by the in-
vading fungus the host develops callus tissue which may partially cover

the injured area. This newly formed tissue is subsequently invaded by the fungus and the concentric ring formation of the cankered area results.
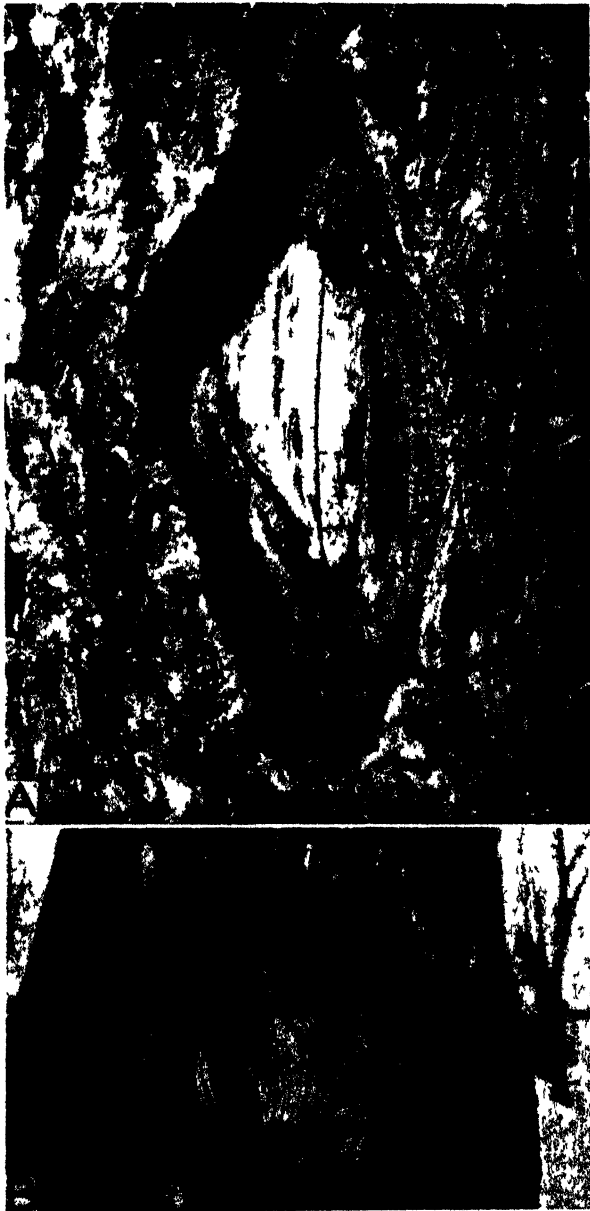
FIG. 2. A. Pit canker development 18 months after inoculation with *Phytophthora inflata* n. sp. Bark has been removed to show the concentric rings formed. B. Old canker showing concentric markings.

Cankers are often masked by the thick bark of the trunk (Fig. 1, B). Bleeding, in the form of a red-brown exudate, often occurs from small fis-

sures near the margin of the cankers. This bleeding is easily distinguished from slime flux in elms which is usually light brown or dark gray. In severely infected trees the trunks become rough and irregular as a result of depressions formed by the cankers and sloughing of the bark near the affected areas. A marked decline of infected trees is indicated by sparse yellow and pale green foliage, reduced terminal twig growth, and, in certain instances, dieback of twigs and small branches.

Internal symptoms are characteristic red-brown discolored tissues in the phloem, the cambium, and the outer area of the xylem. On the trunk where a number of cankers are localized, adjacent cankers are frequently connected by strands of discolored, infected tissue. Where cankers are widely separated there does not appear to be any connection between them.

THE PATHOGEN

Investigations on the disease were initiated in the spring of 1946 when tissues collected from declining trees growing in various sections of Connecticut and Massachusetts yielded a *Phytophthora*. Twenty-five street trees in Greenwich, Connecticut,[3] each with typical cankers, were used as sources of material at intervals through the spring and summer.

On May 5, 1946, samples taken from the 25 diseased trees were cultured on apple-potato-dextrose agar. The organisms that developed from the transplanted tissues were examined periodically. After 14 days only two samples yielded *Phytophthora*. Samples were collected again on June 4, 1946, from those trees which were negative for *Phytophthora*, and four more trees were found infected. On June 22, 1947, three additional cases were found; on September 10, 1947, four cases proved positive and on October 11, 1947, two more trees yielded the pathogen. Finally the *Phytophthora* was isolated from 15 of the 25 cankered trees

Difficulty in isolating the fungus on agar media resulted from the presence of contaminants such as *Alternaria* sp., *Monilinia* sp., *Rhizopus* sp., and *Fusarium* sp. These fungi grew rapidly and often masked the presence of the slow-growing *Phytophthora*. Isolation of *Phytophthora* was most successful when the inoculum was plated on autoclaved applesauce. The samples to be cultured were dipped in alcohol and quickly flamed. Excessive heat was avoided by using rapid recurrent flamings of short duration. After flaming, the samples were placed on autoclaved applesauce in Petri dishes. When a fungus growth was noted during daily examinations with a dissecting microscope, mycelium was transferred to apple-potato-dextrose agar in another Petri dish. The transferred organism was also observed closely and as soon as a coenocytic mycelial strand was observed, it was immediately transferred to potato-dextrose agar. On apple-potato-dextrose agar the fungus develops a compact brown circular pad of mycelium adhering closely to the substrate. This characteristic has aided in iso-

lation, particularly when various contaminants overran the surface of the medium.

Isolates obtained from trees growing in Massachusetts, Connecticut, and New York proved indistinguishable in morphologic and cultural characters. The fungus differs in important respects from previously described species of the genus and is here designated a new species.

Phytophthora inflata sp. nov.[4]

Hyphae continuous when young, becoming septate with age, usually smooth. Sporangia rare on agar media but developing abundantly on washed mycelial mats transferred from 7-day pea broth cultures to sterile distilled water and incubated 7 days; sporangia borne terminally on sporangiophores not differentiated from vegetative hyphae, limoniform to elongate, papilla broad, rounded and nearly filled by contents of sporangium; hyaline dehiscence plug lenticular; sporangia variable in shape and size, 20–67×15–$32\ \mu$, averaging about $38 \times 23\ \mu$. Zoospores fully differentiated within the sporangium, biflagellate, reniform in motile stage, spheroidal in quiescent stage, 10–$12\ \mu$ in diameter. Chlamydospores not observed, but thin-walled, hyaline intercalary hyphal swellings occasionally develop. Oogonia, abundant in oatmeal agar cultures, thin-walled, hyaline, spheroidal, stalk narrow, not infundibuliform, 30–$42.7\ \mu$, averaging $34\ \mu$. Oospores single, spheroidal, smooth, nearly filling oogonium, hyaline to straw color, wall fairly thick (3–$4\ \mu$), contents densely granular, usually with a single large reserve globule and frequently 1 to 4 smaller globules, 26–$39.3\ \mu$, averaging $31.3\ \mu$. Antheridia paragynous, point of contact with oogonium usually near oogonial stalk, inflated, usually variously contorted, often twining or twisted about oogonial stalk, often irregularly lobed or branched, arising from a separate hypha or, occasionally, from oogonial hypha, more numerous in culture than oogonia but seldom more than one in contact with an oogonium, up to $50\ \mu$ long and $15\ \mu$ in diameter.

Habitat—living cortical tissues of *Ulmus americana* L. and *U. fulva* Michx., northeastern United States of America.

Type cultures have been deposited in the American Type Culture Collection and at the Centraalbureau voor Schimmelcultures.

Hyphis primo continuis, maturitate septatis, vulgo levibus.
Sporangiis in agare cultis raris, sed copiosis in mattis mycelialibus lavatis e solutione nutritia septem dierum ad aquam distillatum et sterilem translatis et septum dies incubatis; nascentibus in sporangiophoris non a mycelio distinctis; vulgo limoniformibus, elongatis, papillatis, 20–67×15–32 micra, fere circa 38–23 micra.
Zoosporis in sporangia formatis, biflagellatis, motis concavo-convexis, quietis globosis, in diametrum 10–12 micra.
Chlamydosporis ignotis.
Oogoniis spheroidalibus, hyalinis, levibus, tenuimembraneis, cauli angusto, 30–42.7 micra, fere 34 micra. Oosporibus singulis, levibus spheroidalibus hyalinis aut subfuscis, 26–39.3 micra, fere 31.3 micra. Membrana crassa (3–4 micra). Antheridiis paragynosis, inflatis, vulgo multis modis contortis, non constanter lobatis aut ramosis.

Habitat in cortice vivo *Ulmi americanae* et *U. fulvae* in regione U. S. A. vergente in septentriones et orientem solem.

Phytophthora inflata grows well on the usual agar media. On Difco corn-meal agar (pH 6.0) the temperature-growth relations of the species indicate an optimum temperature between 25° and 30° C. Growth of Petri-plate cultures after 96 hr. was as follows: at 35° C.—no growth, inoculum killed in 96 hr.; at 30° C.—33 mm.; at 25° C.—34 mm.; at 20° C.—29 mm. Oogonia and antheridia develop abundantly on cornmeal agar but are somewhat smaller than those developing on oatmeal agar. The species resembles *P. megasperma* Drech. and *P. erythroseptica* Pethyb. to a degree in the large size of oogonia and oospores. It may be distinguished from *P. erythroseptica* by its paragynous antheridia, and from *P. megasperma*, as from all other species, by the large, inflated, contorted, variously lobed or branched antheridia which are considered the distinguishing character of the species (Fig. 3, 9–17). The antheridia may be observed most favorably in young cultures prior to, or soon after, fertilization of the oogonia. In older cultures they are often tightly twisted around the oogonial stalk and difficult to see.

The character of the sporangia developed in distilled water is not well marked. The broadly rounded papilla with its rather thin or lenticular hyaline plug suggests the non-papillate sporangia characteristic of *Phytophthora cryptogea* Pethyb. & Laff., *P. cinnamomi* Rands, and closely related species. Sporangia developed in oatmeal agar cultures, though few in number, have the well-defined hyaline plug similar to those formed by *P. parasitica* Dast., *P. palmivora* Butl., and *P. capsici* Leon.

In apple fruits inoculated by incision, *Phytophthora inflata* caused a firm, rapidly spreading brown rot. Approximately half of a potato tuber was invaded by the fungus within one week. When the invaded potato tissue was exposed to the air a bright pink color developed within a few minutes. The brown rot of apple fruits and the pink rot of potato tubers were similar to rots caused by numerous species of the genus (5).

PATHOGENICITY TESTS

The pathogenicity of the *Phytophthora* was tested on 27 elms ranging from 3 to 17 in. in diameter and growing on low wet and on high dry sites. Inoculations were made by inserting mycelium from active cultures into vertical wounds made in the trunks with a flamed scalpel. Sterile media and the fungi mentioned previously as contaminants were placed in wounds on trees as a check on symptom development. In 1946, five trees were inoculated on August 5; five on August 20; four on September 15; nine on October 15; and four on October 22. About five weeks after the first three groups were inoculated, symptoms began to appear in 81 per cent of the trees, while the checks remained apparently healthy. The first noticeable symptom was the presence of a soft, dark brown exudate near the point of inoculation. As the season progressed the underlying tissue became ne-
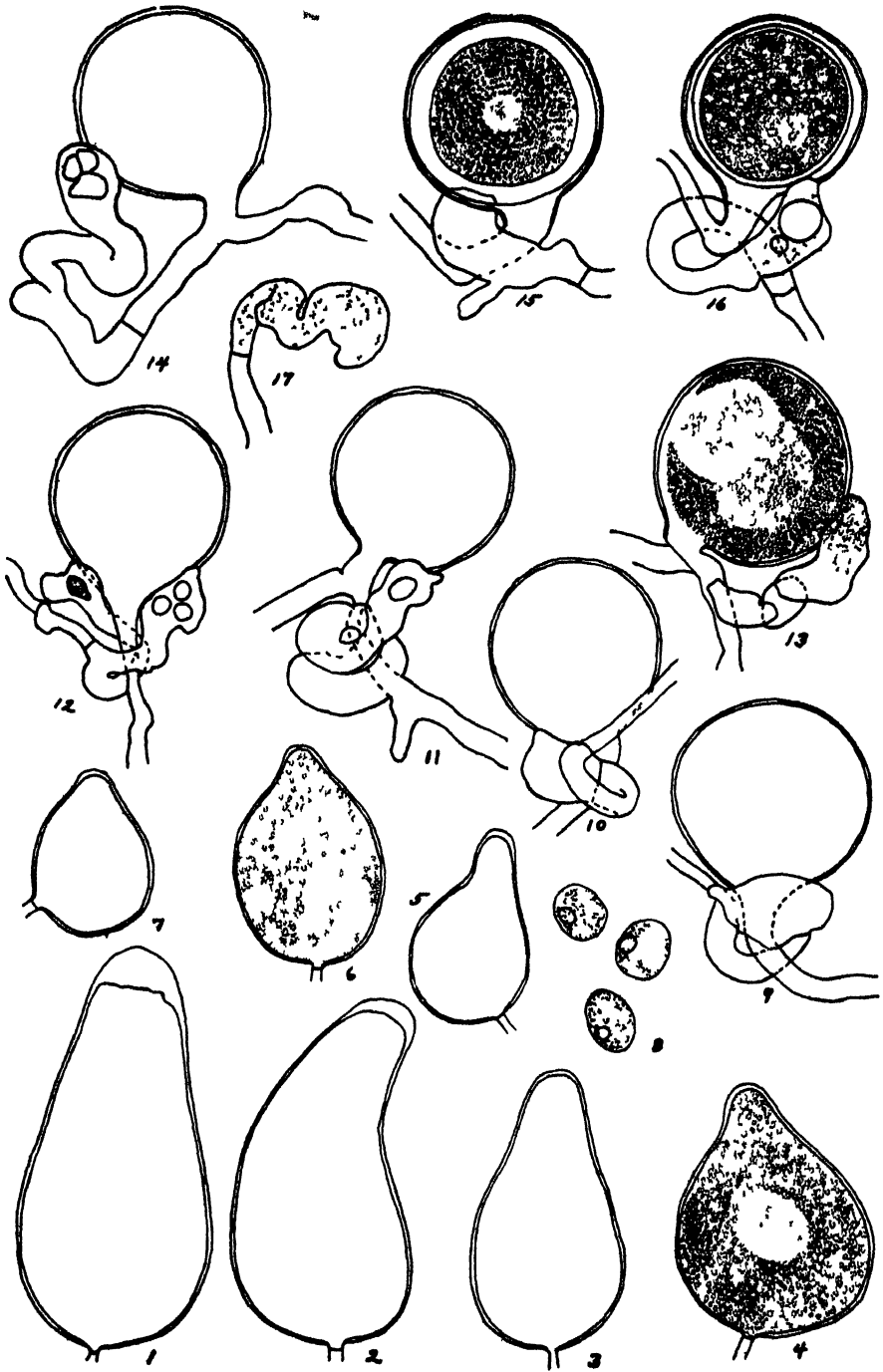
FIG. 3.   Phytophthora inflata n. sp.   1-7, Sporangia developed on washed mycelium transferred from pea broth to distilled water; 8, zoospores; 9-14, oogonia and antheridia; 15, 16, oogonia, oospores, and antheridia; 17, antheridium.   (× 1000)

crotic and cankers developed similar to those from which the fungus was isolated. As new tissue developed, it was killed back by the fungus. This produced the concentric zoning characteristic of the cankers (Fig. 2, A). In the spring of 1947, 83 per cent of the inoculated trees had symptoms of canker. Removal of the bark of the newly formed cankers showed that the infected tissues were immediately adjacent to the cambium and were dark brown as in naturally infected trees.

Reisolation from diseased tissues at some distance from the point of inoculation consistently yielded a *Phytophthora* identical with the isolate used for inoculation.

SUMMARY

Pit canker of the American elm occurs widely distributed in New York, Pennsylvania, Connecticut, and Massachusetts.

Phytophthora inflata n. sp. was associated with the cankers. The causal relationship of the fungus was established according to Koch's postulates.

Phytophthora inflata n. sp. is readily identified in culture by the development of large, inflated antheridia, which are usually variously contorted, lobed, or branched.

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FORMATION OF GALLS IN STEMS AND LEAVES OF SUGAR CANE IN RESPONSE TO INJECTIONS OF GROWTH-REGULATING SUBSTANCES[1]

J . G U I S C A F R É - A R R I L L A G A

During the late summer and fall of 1947 and 1948, experiments were undertaken at Baton Rouge, Louisiana, to test the effect of various growth-regulating substances upon the inducement of floral organs in sugar cane. Sugar cane seldom blooms in this area, and when it has bloomed, only a few tassels have formed in a whole field, thus indicating that conditions in this region are not quite favorable for flowering.

Naphthalene-acetic acid, indole-acetic acid, and 2,4-dichlorophenoxyacetic acid were injected approximately $\frac{1}{2}$ in. above and $\frac{1}{2}$ in. below the terminal bud in amounts of 0.5, 1.0, 1.5, 3.0, and 4.0 mg. Solutions of these chemicals were prepared so as to contain 1 mg. per cc. Concentrations used were based on those found by Van Overbeek (8) to induce flower formation in the pineapple plant. Ten stalks of C.P. 29/116 were injected with different amounts of each of the three chemicals. Stalks of this variety were also injected with 0.5 cc. of a solution containing 0.5 mg. of naphthalene acetic acid and 0.5 mg. of colchicine per cc. Similar tests were conducted in the same field at a distance of 200 yd. using the variety C.P. 29/320. In both cases, plants were treated also with 0.5, 1, and 1.5 cc. of sterilized distilled water. To serve as controls, a number were punctured with the needle and air was blown into the tissues. Tests were started on August 15. Different lots of stalks were injected with varying dosages of each chemical. The concentrations of 1.0 and 1.5 mg. were divided for some lots into two or three portions of 0.5 mg. each and injected at 2-wk. intervals. For amounts of solution such as 0.5 and 1.0 cc., the short type 1-cc. Yale Insulin Hypodermic Syringe No. 1Y1–80 with needle size Bd 27 was used; for larger quantities, the 5 cc. B-D Yale Hypodermic Syringe No. 5Y.

The needle was thrust at an angle of 30–40° until it had penetrated through half of the diameter of the stalk at the region where the terminal bud was located. The liquid could not be forced into the tissue unless the needle was drawn out about 2 mm. It often was necessary to free the needle of plugging material after each injection. Both needle and syringe were thoroughly cleaned several times by flushing with 95 per cent ethyl alcohol and sterilized distilled water, when changing to another chemical.

With the exception of observations on the technique of treatment, no

FIG. 1 Single stalk on left· sprouting of lateral buds induced by injecting 3–5 mg. of indole acetic acid. Three stalks on right: galls induced by injecting stalks with 0 5 mg. of indole acetic acid three times at intervals of 2 weeks.

results were obtained from the 1947 experiments, yet it proved feasible to introduce as much as 5 cc. of liquid near the terminal bud without apparent injury. Further observations were not possible because the lot was har-

vested before any reasonable length of time had elapsed after injection of the chemicals. Observations made between November 1 and December 31, 1948, showed no initiation of floral organs in any of the stalks treated.

However, some very interesting responses to the injection of various amounts of the growth-regulating substances at the terminal bud were observed. With larger amounts of indole-acetic acid such as 3 and 4 mg., the newly formed internodes grew at an angle in a zigzag fashion and the buds sprouted into shoots, the terminal bud remaining unaffected.
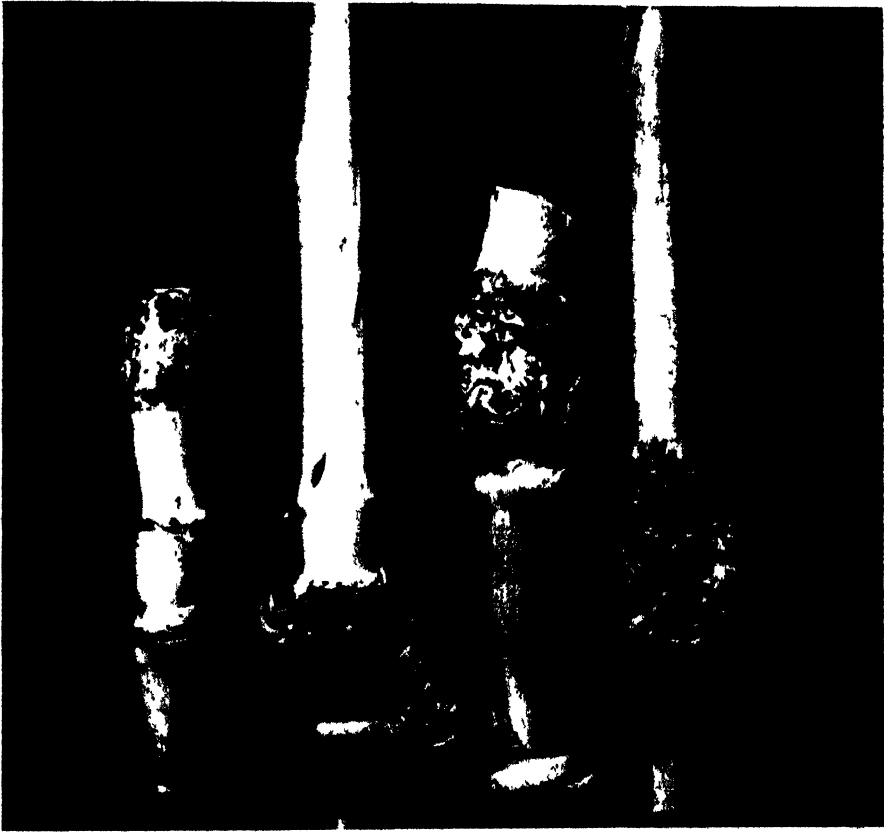
FIG. 2. Two stalks on left: gall formation induced by injection of 0.5 mg. of 2,4-D. Two stalks on right: gall formation induced by injection of a mixture of 0.5 mg. of naphthalene acetic acid and 0.5 mg. of colchicine.

Amounts of indole-acetic acid corresponding to 1.5 mg. divided into 3 portions of 0.5 mg. each and injected at 2-wk. intervals caused the formation of galls in the more mature internodes close to the top of the stalks. These galls were formed both at the nodes and on the internodes and gave origin to buds growing in all directions, both at the internodal and nodal regions (Fig. 1). These galls were not of a folioid nature but rather firm, pinkish, and bud-like. Out of 10 stalks thus treated, 5 showed these galls profusely, while other stalks in the vicinity, and some originating from the

same clump but treated differently or receiving no treatment at all, had no gall formation. The galls resembled those on sugar cane as reported by Lyon (3), Martin (4, 5), and Tims (7).

When applied in amounts of 0.5 mg., 2,4-dichlorophenoxyacetic acid induced gall formation at the base of the youngest leaves visible in the spindle and in the covered immature internodes. The galls in the leaves were small and scabby, while those in the covered immature internodes were large, tender, and distinctly folioid (Fig. 2). When mixed in the same solution, 0.5 mg. each of naphthalene acetic acid and colchicine induced the same type of folioid galls in covered immature internodes in 4 of the 10 stalks treated (Fig. 2).

None of the responses described was observed in any of the other treatments nor in stalks injected with water, or simply punctured or not treated at all. Untreated stalks of other varieties in the vicinity of the treated plots showed no gall formations. The infestation of the borer was great in all the plots and to the same extent in treated and nontreated stalks.

Galls have been observed in sugar cane by several investigators including Kamerling (2), Barnum (1), Lyon (3), Martin (4, 5), and Tims (7). Tims (7) obtained galls in sugar cane by planting cuttings from stalks of P.O.J. 234 previously showing gall formation or showing symptoms of the dwarf or multiple bud disease. Martin (5, 6) reported the inducement of gall formation in sugar cane by injections of extracts obtained from crushed green leafhopper, *Draeculacephala mollipes*, corn leafhopper, *Peregrinus maidis*, and pink sugar cane mealy bug, *Tryonymus sacchari*. He suggested that the green leafhopper as well as the other insects might carry certain auxins or growth-promoting substances which induced gall formation.

The findings hereby reported support Martin's (5, 6) observations and theory as to the etiology of galls in sugar cane as well as that of Went and Thimann (9) on the cause of pathological outgrowths of plants as being traceable to auxin effects.

This seems to be the first instance in which galls have been induced in sugar cane by the artificial injection of growth-regulating substances, and offers a new and practical method of stimulating the formation of excessive proliferated tissues which may give origin to polyploid plants.

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REPORT OF THE 1949 ANNUAL MEETING OF THE SOUTHERN DIVISION, THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The 1949 annual meeting of the Southern Division of The American Phytopathological Society coincided with that of the Association of Southern Agricultural Workers, January 31 through February 2, 1949, at Baton Rouge, Louisiana. More than 90 plant pathologists were in attendance. In addition to the 34 papers presented, a round-table discussion on the plant nematode problems of the South was held, led by Dr. G. Steiner of Beltsville, Maryland.

Officers elected for 1949–50 are: *President*, L. M. BLANK; *Vice-President*, W. A. JENKINS; *Secretary-Treasurer*, J. A. LYLE; *Councilor*, A. L. SMITH.

Titles and abstracts of papers presented follow.

J. A. LYLE, *Secretary-Treasurer*

Fungicides and Fall Cucumbers in Louisiana. ATKINS, J. G., JR. In Louisiana the basis for evaluation in the testing of fungicides for fall cucumbers has shifted in recent years from one based upon the control of downy mildew to one based upon the control of two foliage diseases, downy mildew and anthracnose. Although downy mildew still ranks as the principal foliage disease, the frequent severity of anthracnose necessitates a fungicide effective against both diseases. The testing of organic and fixed copper fungicides has been complicated by the varying severity of anthracnose from year to year. In 1947, a year of heavy anthracnose infection, Tri-Basic Copper Sulphate gave poor performance as a cucumber fungicide in comparison with the organic fungicides. However, the same fungicide gave good results in 1948 when anthracnose was not severe. In both the 1947 and 1948 tests Dithane Z-78 (zinc ethylene bisdithiocarbamate), Fermate (ferric dimethyl dithiocarbamate), Zerlate (zinc dimethyl dithiocarbamate), and Parzate (zinc ethylene bisdithiocarbamate) gave good control of both downy mildew and anthracnose. Dithane Z-78 was not phytotoxic on cucumbers but Parzate caused considerable injury.

Varietal Response of Snap Beans to Seed Treatment. BAIN, DOUGLAS C. Studies of seed treatments for control of seed-rot and damping-off of snap beans were made in the field at Crystal Springs, Mississippi. The materials used were Spergon (98 per cent tetrachloro-para-benzoquinone), Arasan (50 per cent tetramethyl thiuramdisulfide), Arasan SF (75 per cent tetramethyl thiuramdisulfide), Ceresan M (ethyl mercury p-toluene sulfonanilide), Semesan (30 per cent hydroxymercurichlorophenol), Puratized 177 (phenyl mercury triethanolammonium lactate), and 2 per cent Ceresan (2 per cent ethyl mercury chloride). The materials were applied as dust or slurry or both. Plantings were made in both spring and late summer. Results indicated that in spring planting the variety Stringless Black Valentine responded significantly to some treatments in increase of emergence, stand, and yield; however, under like conditions Fulgreen 1 responded only slightly. In late summer plantings of Stringless Black Valentine there was a significant increase in emergence and stand but not in yield; the response of the U. S. Dept. Agr. line B1625-17, however, was negative; i.e., emergence, stand, and yield were reduced in most cases, while plants from untreated seed had the highest yield. These differences in response did not appear to be due to resistance to seed-rotting and damping-off organisms, if the percentage of loss due to these causes is used as the criterion. Certain treatments among these varieties appeared to increase emergence and stand but decreased yield as compared with untreated seed.

Breeding for Resistance to Bacterial Blight of Cotton. BLANK, LESTER M. Stoneville 20 strain of cotton has been used as the blight resistant parent in a number of crosses with susceptible varieties and breeding strains of cotton. The resulting progenies were carried through the F$_3$ generation or have been backcrossed twice to the recurrent susceptible variety or strain, selfed, and tested for disease reaction. Inoculation of the breeding material in field plantings was accomplished by spraying the underside of leaves of 5- to 8-week-old plants with a suspension of *Xanthomonas malvacearum*.

Grading of plants as to disease reaction was made 15 to 20 days later. Resistant pheno-types have fewer and smaller lesions than do susceptible phenotypes. F_1 progenies of the original cross were susceptible while F_2 progenies segregated at a ratio approxi-mating 3:1 for susceptibility and resistance. This ratio was realized most closely in crosses involving highly susceptible varieties such as Acala, and less closely in crosses involving varieties having considerable natural tolerance. Progenies derived from selfing after one and two backcrosses gave populations which were pure for susceptibility or were segregating, the segregating populations having susceptible and resistant plants in a ratio of 3:1. These results suggest that the Stoneville 20 type of resistance to bacterial blight is inherited as a single factor, with resistance recessive to susceptibility.

Hypodermic Injection as a Method of Inoculating Cotton Plants with Verticillium albo-atrum. BRINKERHOFF, L. A. In breeding for resistance to Verticillium wilt of cotton much time is usually spent selfing plants that prove to be susceptible later in the season. In California and Arizona rarely more than 15 to 30 per cent of a susceptible population will show symptoms at the start of the selfing season. This is true for both naturally infested or artificially inoculated soil. Hypodermic injection and wound in-oculation both produced high percentages of infection in young field-grown cotton plants in 1947 and 1948. The injection method proved to be about as rapid as soil inoculation and much faster than wound inoculation. Seedlings with two to four true leaves were much easier to inject than older plants. A veterinary syringe with a No. 24 needle was used to inject approximately ¼ ml. of inoculum into the hypocotyl of young seedlings just below the soil level. Ninety-nine and 98 per cent respectively of a susceptible variety, Acala 1–23–11, and a tolerant variety, Missdel × Acala 10–13, developed symp-toms within 3 weeks after injection on May 17, at Shafter, California. The tolerant variety was much less severely infected, and a much higher percentage of the plants re-covered from the disease.

Induced Variability in Phoma lingam. CALVERT, O. H., GLENN S. POUND, J. C. WALKER, MARK A. STAHMANN, and J. F. STAUFFER, JR. Induced variability of the Puget Sound strain of *Phoma lingam* recently described as occurring on cabbage seed plants has been studied in relation to a sudden development of seed transmission on Puget Sound seed. In determining whether this blackleg epidemic resulted from in-creased activity of the Puget Sound strain, nitrogen mustard (methyl-bis (B-chloro-ethyl) amine) was used to induce cultural and pathogenic variability. It appeared that the rate of mutation could be markedly increased by appropriate treatment and the nitrogen mustard appeared to be the most effective means used by which mutations could be induced. In one experiment over 60 per cent of mutations occurred among surviving spores. There was apparently no correlation between the type of mutant produced and the method of treatment used. Greenhouse inoculations were made to determine the extent of induced pathogenic variability by nitrogen mustard. No isolate produced lesions characteristic of those of the eastern type strains, but evidence was presented to show that through increased virulence some of the isolates were able to produce a disease as severe as that caused by the eastern strains. Complete evidence was not found, however, that would explain the sudden development of blackleg transmission on western-grown seed.

The Nature of Resistance in Plants to Root-Knot. CHRISTIE, J. R. The root-knot nematode, *Heterodera marioni*, is a sedentary parasite. It remains in the same loca-tion throughout the parasitic phase of its life and feeds by puncturing cells within reach of its head and sucking out their content. In a suitable host, pronounced mor-phological and physiological changes in the surrounding plant tissues, including the formation of so-called ''giant cells,'' provide the parasite with a continuing source of accessible food. It is suggested that, in the case of resistant plants whose roots are invaded but in which larvae fail to develop, changes in the surrounding plant tissues, necessary for the development of the parasite, fail to take place and the nematode dies of starvation.

The So-Called Live-Oak Disease in Texas. DUNLAP, A. A., and A. L. HARRISON. The dying of live oaks in certain areas of Texas has become a serious problem for home owners and ranchmen. Both large and small trees die at different seasons and under a wide variety of environmental conditions. As yet, no causal agent has been identified in connection with this trouble, which is presumed to be some sort of disease. Timely, severe pruning of all large branches at the first appearance of the trouble has resulted in apparent recovery and renewed growth from the main trunk of the tree.

Fungicides for the Control of Raspberry Diseases. EPPS, JAMES M., and C. D. SHERBAKOFF. Of several fungicides tested for the control of raspberry diseases, Fer-

mate (ferric dimethyl dithiocarbamate) proved most effective when used at the rate of 2 lb. in 100 gal. of water. A delayed dormant spray of 1 to 10 lime-sulphur applied at the time the buds are breaking and three applications of the Fermate spray gave a substantial increase in yield over the other treatments. The copper sprays and dusts did not give an increase in yield over the plot that received the delayed dormant spray alone.

Pecan Scab Spray Experiments in South Carolina During 1948. FOSTER, H. H., A. M. MUSSER, R. J. HIGDON, and M. B. HUGHES. Experimental spray plots, using the scab (*Cladosporium effusum*) susceptible Schley variety, were located near Orangeburg and at the Edisto Experiment Station. Bordeaux Mixture (4–1–100) for first cover spray, and (6–2–100) for second, third, and fourth cover sprays, Zerlate and Karbam White (zinc dimethyl dithiocarbamate) (2 lb.–100), were used at the Edisto Station. At the Orangeburg plots Bordeaux Mixture, Copper-A (3lb.–100), Zerlate, Parzate (zinc ethylene bis dithiocarbamate) (2 lb.–100), and a Cu.-Zn. compound, mixed for pecan spraying (5 lb.–100), were used. Scab infection was rated from one (no infection) to five (numerous secondary infections). Unsprayed check trees showed 77 to 99 per cent of nuts examined to be severely scabbed (class 5). At the Edisto Station Bordeaux Mixture gave practical control of scab and increased the size and weight of nuts, while Zerlate and Karbam White failed to give satisfactory scab control. At the Orangeburg plots all sprays except the Cu.-Zn. compound gave practical scab control and increased the size and weight of nuts. Zerlate was superior to other treatments. At the Orangeburg plots, in part because of insufficient rainfall, some nuts from all treatments including the control had split shells. Bordeaux-sprayed nuts had more split shells than did those under other treatments.

Fungicidal Control of Cercospora Blight of Pepper. HARE, W. W., D. C. BAIN, and T. E. ASHLEY. A field of pepper almost completely defoliated by *Cercospora capsici* was found in the canning area near Wiggins, Mississippi, on July 23, 1948. Spray and dust treatments were applied in replicated plots in the field on that date and approximately every 2 weeks afterward until the end of September. One month after final application a disease rating of the plants was made, based on the percentage of defoliation of the second crop of leaves. Analysis of indexes calculated for each treatment plot showed highly significant effects of Bordeaux (4–4–50) and Copper A sprays as compared with the dusts in the prevention of defoliation. Copper A and Tribasic Copper dusts were highly significantly better than the checks. There were no significant differences between the two sprays or the two dusts. The sprays gave satisfactory control of the disease. The control obtained with the dusts was not considered sufficient for practical use.

Some Factors Affecting Viability of Peanut Seed. HIGGINS, B. B. Most of the late-harvested peanuts of the 1947 crop in the southeastern area of the United States were severely weather-damaged; consequently, the viability was low. Stands obtained from early plantings were generally poor. In an attempt to clear up some questions as to the effect of various chemical seed-treatment materials, variation in rate of application, and of weather conditions, tests were set up using farmers' stock Runner and Spanish peanuts which were machine-shelled and treated with Dow 9B (50 per cent zinc trichlorophenate), 2 per cent Ceresan (2 per cent ethyl mercury chloride), Arasan (50 per cent tetramethyl thiuramdisulfide), or Spergon (98 per cent tetrachloro-para-benzoquinone), all at normal, medium-high, and high rates of application and with machine-shelled and hand-shelled checks. Two plantings of the Runner seed were made, one when conditions for germination were nearly ideal and the other just before a series of heavy rains. The Spanish were planted under nearly ideal conditions for germination. Both Runner and Spanish were low in viability and both were severely damaged by machine shelling. There was no noticeable indication of injury from the highest rate of application (6 oz. to 100 lb. of seed) for any of the chemicals. At corresponding rates of application, 2 per cent Ceresan was significantly superior to all other materials, except at 6 oz. to 100 lb. of Spanish seed when the superiority to Arasan and Spergon was not significant. The Runner seed planted just before a series of heavy rains lost more than 32 per cent in viability. Studies and observations for several years indicate that this may be due to loss of a substance from the seed coat, probably the pigment, which appears to have antioxidant and possibly fungistatic properties.

Baldulus Maidis, Leafhopper Vector of Corn Stunt Virus, in Texas. HILDEBRAND, E. M. Adult, light-colored leafhoppers collected near Weslaco, Texas, on May 1, 1948, were placed on small caged field-corn plants for study at College Station. Within 4

days wilt-blight necrotic symptoms developed on the small plants receiving the light-colored leafhoppers, whereas no symptoms developed on plants receiving darker colored leafhoppers. The symptomed plants survived when the leafhoppers were promptly removed. On the 5th day all of the latter (dark-colored) leafhoppers were dead whereas two of the former were alive; these were transferred to healthy caged corn seedlings. On the 6th day some early instar nymphs were found on the seedlings first fed on by the leafhoppers. By the 22nd day the first adults appeared and on the 24th numerous adults were present. Thus the life cycle had been completed within about 3 weeks. Adult specimens of the first brood were identified by Oman as *Baldulus Maidis*. Transfers of leafhoppers were made successively at short intervals to small seedlings for about 3 months. The adult-to-adult leafhopper generation period ranged between 18 and 24 days. Essentially the same generation time obtained for leafhoppers present on diseased plants. The shortest incubation interval between feeding on diseased corn and actual transmission was approximately 24 days.

Fungus Gall on Jasmine. HILDEBRAND, E. M. Gall specimens simulating crown gall on above-ground stems of Winter Jasmine, *Jasmium nudiflorum*, were received from Arlington in October, 1948, and isolations were made. The interiors were sterile whereas the surface tissues yielded a fungus by both tissue-plating and dilution techniques. Abundant black pycnidia, measuring 77 to 98 μ in diameter, covered the gall surface. The radial extension of mature galls ranged between 6 and 12 mm. The fawn-gray cultures produced pycnidia on potato-dextrose agar. Spray inoculation of wounded and unwounded plants of two species of Jasmine and Privet resulted in most abundant infections on wounded plants receiving a post-inoculation high-humidity treatment. Simple wound inoculations were also successful. Gall symptoms were appearing within 2 weeks, with radial extensions of about 1.5 mm. at 1 month and 3 mm. at 2 months. These Jasmine galls were very similar to the so-called Phomopsis galls reported by Brown from Maryland in 1936 and observed by the writer in New York on wild rose in 1937 and on forsythia in 1938. Pycnidiospores are very small, about 3.4 × 1.5 μ. Based on its small spore size and its lack of *b* spores, this fungus would appear to be a new *Phoma* species.

Grafting Methods Used in Attempts to Transmit the Littleleaf Disease. JACKSON, L. W. R., and BRATISLAV ZAK. The systemic nature of the littleleaf disease of short-leaf pine (*Pinus echinata*) suggested that it may be caused by a transmissible virus. The transmissibility of littleleaf was tested by grafts made on seedling, sapling, and adult shortleaf pines. The grafting methods are described and illustrated. Stem, branch, and root parts were successfully grafted by the bark patch and the approach graft methods. Much of the difficulty involved in grafting was due to the age of the scion and stock material, because the littleleaf disease does not affect trees under 20 years of age. Positive fusions with bark patch grafts in all except 3 of the special types of grafts ranged from 19 to 100 per cent. Bark patch grafts made with a thin layer of underlying wood or with attached twigs were not successful. Positive fusions with seedling stems grafted on stems of diseased and healthy trees were 78 and 83 per cent, respectively. Positive fusions with seedling roots grafted on roots of diseased and healthy trees were 55 and 36 per cent, respectively. Thus far, the various types of grafts have failed to demonstrate that the littleleaf disease is caused by a virus.

Pathogenicity Studies with Colletotrichum Isolates from Sugar Cane, Sorghum, and Other Crosses. LEREAU, F. J. Three hundred and seventy-six isolations of *Colletotrichum* from 17 different hosts, the majority coming from sugar cane, sorghum, Johnson grass, Sudan grass, and *Erianthus sp.*, have been tested for pathogenicity towards sugar cane (red rot in variety C. P. 29/116) and sorghum (leaf spot on variety Collier). Of these, 50 cultures were also tested for ability to produce stalk rot in sorghum variety Rex growing in the field. An additional 197 cultures from Johnson grass, sorghum, and sugar cane were tested for their ability to produce stalk rot in sugar cane and sorghum. Using disease ratings from 0 to 4, with 4 indicating greatest severity of disease, the average ratings were 3.58, 1.55, 2.17, 1.46, and 1.78 for sugar cane inoculated with cultures from sugar cane, sorghum, Johnson grass, *Erianthus sp.*, and Sudan grass, respectively. Punctured noninoculated controls yielded an index of 1.11. Sorghum seedlings, inoculated in the leaf whorl with cultures from sugar cane, sorghum, Johnson grass, *Erianthus sp.*, and Sudan grass yielded average ratings of 1.79, 3.44, 2.96, 3.16, and 3.11, respectively. In standing Rex sorghum, isolates from sugar cane, sorghum, and *Erianthus sp.* produced ratings of 1.91, 2.80, and 2.49, respectively. In cut Rex sorghum, cultures from sugar cane, sorghum, and Johnson grass yielded ratings of 1.88, 3.47, and 3.47, respectively. Punctured controls yielded an index of 1.17. Thus the Colletotrichum population on sugar cane is different from that affecting sorghum, Johnson grass, *Erianthus sp.*, and Sudan grass.

Soil Fumigation for the Control of the Root-Knot Nematode in Peach, Fig, and Grape Plantings. MACHMER, JOHN H. Planting sites at Tifton, Georgia, fumigated in January, 1944, at 6- and 18-in. depths totaling 78 cc. chloropicrin in 26 injections per site were planted in February with Elberta peach trees averaging minus 0.39-in. trunk diameters. The first season's average trunk diameter increases were 1.05 and 0.72 in. with root-knot-resistant and susceptible cover crops respectively. Nonfumed increases were only 0.60 and 0.47 in. per respective cover. After 5 seasons, average trunks on fumed sites with resistant cover measured 3.88 in. and limbs occupied 802 cu. ft. of spread. Trunks on nonfumed sites with susceptible cover measured 2.59 in. and limbs occupied 409 cu. ft. Including 1948 yields, average trees on fumed sites produced 46.4 lb. and 23.9 lb. fruit with resistant and susceptible cover respectively. Trees on nonfumed sites produced 18.2 lb. and 9.7 lb. fruit with respective covers. Average trees on sites fumigated deep and shallow with 210 cc. chloropicrin in 70 injections and grown with root-knot-resistant cover 4 seasons produced 1242 cu. ft. limb spread and 4.49 in. trunk diameters compared with 538 cu. ft. and 3.01 in. on nonfumed sites. Fumigating subsoil produced greater tree vigor than fumigating larger shallow areas. Similar results obtained with fig and grape.

The Status of Phymatotrichum Root Rot of Cotton in Louisiana in 1948. NEAL, D. C. Cotton root rot caused by the fungus *Phymatotrichum omnivorum* was first reported in Louisiana in 1947. At that time two infested localities were located in the Red River alluvial area. One is in Bossier Parish embracing two separate fields of approximately 30 acres, and the other in Caddo Parish consisting of about 4.5 acres. These two parishes were surveyed again during the past season and two new infested localities were found. One consists of only a small spot roughly 35 × 50 feet in Bossier Parish and located 1.5 mi. north of the original 1947 infested fields. The other is in Caddo Parish and consists of approximately 13 acres located about 8 mi. northwest of the first field found infested in 1947. The points of interest concerning the prevalence of the disease in Louisiana are that it is confined entirely to the Red River cotton area bordering Arkansas and Texas; and that among the 4 localities known to be infested, one small spot was found in 1948 which did not occur in 1947 or was overlooked when that survey was made. The occurrence of the disease in a fertile basin both to the east and west of Red River and in fairly close proximity thereto suggests the possibility of its introduction by floods.

Phytophthora cinnamoni and Ramularia sp. Pathogenic on Camellia japonica. PLAKIDAS, A. G. *Phytophthora cinnamomi* Rands and an apparently undescribed species of *Ramularia* have been repeatedly isolated from diseased roots of sickly or dying camellias. Two year old camellias, var. Sarah Frost, with balled roots, were transplanted in 2-gal. crocks in nonsterilized compost soil infested with pure cultures of the two fungi, alone and in combination. *Ramularia* by itself proved mildly parasitic, causing a slight reduction in growth. In the Phytophthora series, 1 of 12 plants was killed; and in the series inoculated with both fungi, 4 of the 21 were killed. In each series, the remaining plants were sickly, making hardly any new growth during the season. Rooted camellia cuttings were planted in chloropicrin-sterilized compost soil infested with pure cultures of the two fungi separately and in combination. The roots of the cuttings were dipped in water suspensions of the fungi before planting. All the plants thus treated were killed within 2 weeks. A new lot of rooted cuttings was planted in the same soil, but without dipping their roots in fungus suspensions. By the end of the summer, 7 of 14 plants in the Phytophthora series, 11 of 20 in the Phytophthora + Ramularia series, and 1 of 14 in the Ramularia series were killed. All the check plants lived and grew vigorously. Of the surviving plants, those inoculated with both fungi were the least thrifty. A synergistic action between *Phytophthora* and *Ramularia* was apparent in both experiments.

Witch's Broom, a Graft-Transmissible Disease of Arizona Ash (Fraxinus berlandieri). PLAKIDAS. A. G. A disease characterized by yellowish leaves about ¼ to ⅓ natural size and multiple, spindly, wirelike shoots was observed in September, 1943, on a tree about 20 years old on the Louisiana State University campus. Of six main branches, all rising from a common crotch, one was completely diseased to the top, four had scattered diseased shoots about halfway to the top, and one was normal. The diseased foliage persisted into late fall long after the healthy leaves were shed. Cuttings from the diseased tree failed to root; those from healthy trees rooted readily. Three young healthy trees in 12-in. pots were grafted in August, 1947, by in-arching diseased shoots, the cut ends of which were kept in water. Two of the grafts made union. The only symptom developing the first season was a pale-green color of leaves, compared with the dark green of the check. In the following season, however, the grafted

trees made only feeble growth, with weak shoots 1 to 3 in. long bearing yellowish small leaves. There was considerable dieback. One of the two grafted trees, after putting out vestigial growth with minute yellow leaves from several buds, died back to the ground. The check trees have grown to a height of 4 to 6 ft. Most of the original tree was dead by 1947 and it was cut down. This apparently lethal disease has been found on other trees of the same species.

Effect of Calcium on the Development of Cotton Fiber, A Preliminary Report. PRESLEY, JOHN T. Studies were made on the effect of varying amounts of calcium on the development of cotton fibers. Plants of the Stoneville 2B variety were grown in washed sand with nutrients added in the form of a complete nutrient solution. The amount of calcium in the nutrient solution was varied from 10 p.p.m. to 250 p.p.m. Fiber produced by the plants at different levels of calcium was analyzed for length, strength, fineness, maturity counts, X-ray angle, and D. P. (degree of polymerization). Plants with abundant calcium produced fiber that was longer, stronger, and higher in maturity count than that produced by calcium-deficient plants. The X-ray angles decreased as the calcium supply decreased.

Report of Sub-Committee Chairman on Verticillium Wilt for 1948. PRESLEY JOHN T. Verticillium wilt is now known to occur over the entire Cotton Belt of the United States from the Atlantic to the Pacific. Losses from the disease, although variable from year to year in any one particular locality, appear to be constantly increasing. The continued increase and spread of Verticillium wilt in cotton has brought about an intensification of efforts to produce a Verticillium-wilt-resistant variety. Considerable breeding work is being done in an effort to introduce resistance to Verticillium wilt into commercially acceptable varieties, and, also, selections are being made from existing varieties in the hope of obtaining tolerant strains. Workers in California, Arizona, New Mexico, Oklahoma, Mississippi, and South Carolina are so engaged.

Types of Nematode Injury on Small-Grain Seedlings. ROSEN, H. R. On seedlings of oats, wheat, barley, and rye representing fall-sown crops, two different groups of symptoms associated with nematodes have been found in Arkansas, particularly in years when the summer and fall seasons were exceptionally dry. One group of symptoms appears to be associated with the typical root-knot nematode and primarily involves swellings, mainly on the lateral seminal roots. The tops of such infected seedlings consist of dull grayish-green leaves which gradually turn yellow, the seed leaves being usually the first to show marked chlorosis. Another group of symptoms consists of localized rotting of seminal roots, often of the subcoronal internode, and a yellowing of leaves. When this internode is rotted through before coronal roots appear, the seedling dies and in some fields a 10 per cent reduction in stand has been estimated as the result of such rotting. In most instances the seedling recovers as the coronal roots develop and the final grain yield may show little ill effect. However, there appears at times considerable reduction in available winter pasturage. G. Steiner has identified *Aphelenchoides*, spp., *Paraphelenchus* spp., and a number of other nematode species in such rotted seminal roots and coronal internodes, but no meadow nematodes.

Soil Fumigants for Controlling Fusarium Wilt and Nematodes of Cotton. SMITH, ALBERT L. DD (Dichloropropane and Dichloropropene) and Isobrome D (23 per cent ethylene dibromide by weight) were compared in row applications at 3.5, 7, and 14 gal. per acre. The 3.5-gal. rate for DD increased the lint yield 45 per cent and controlled wilt satisfactorily. This rate appeared to be the most economical when used in combination with a resistant variety. Approximately 10 gal. of Isobrome D gave comparable results. With planting and treatment performed simultaneously DD reduced emergence at all rates, but emergence was not affected by Isobrome D. At the 14 gal. rate for DD, abnormal plant type and growth resulted, presumably from nitrogen stimulation as a result of treatment.

Histological Studies of Bacterial Blight Infection of the Cotton Plant. THIERS, HARRY D., and LESTER M. BLANK. Histological studies were made of bacterial blight infection of leaves and floral bracts of susceptible and resistant varieties of cotton. Vascular and mesophyll tissues possessing characteristic disease symptoms in different developmental stages were examined. The bacteria enter the leaf through the stoma and become massed in the intercellular spaces of the spongy mesophyll. Cell walls of the tissue are destroyed and the cellular contents liberated, producing watersoaked lesions on the lower surface of the leaf. The bacteria then attack the palisade cells and cause destruction of that tissue indicated by the appearance of similar lesions on the upper surface of the leaf. Bacteria were not observed in the xylem and phloem

but were observed in the parenchymatous sheath surrounding these vascular elements. Floral bract tissue appeared to be more extensively damaged than leaf tissue. The bacteria occurred in greater numbers and the damage was more extensive in lesions on susceptible varieties than on resistant strains. However, the method of tissue destruction appeared similar in both types of host. No morphological barrier was observed which might restrict development of the disease, suggesting that resistance is probably due to physiological incompatibility of host and parasite.

Virus Diseases of Shallot in Louisiana. TIMS, E. C. There are at least two distinct virus diseases affecting the shallot in Louisiana. A virus known as aster yellows has been in the State for at least 10 years. Shallots affected with this virus have a bright yellow color that is distinct from that of other diseases. Aster yellows does not spread rapidly in the field, and is not a very serious disease at the present time. Yellow dwarf is another virus disease that was recognized somewhat later than aster yellows. It causes a mottling, curling, and twisting of the leaves along with marked stunting of the affected plants. This virus spreads rapidly in the field and causes serious losses. It is transmitted by aphids in the field, and is easily transmissible by artificial means. Yield tests for 3 years showed that yellow dwarf reduced the green weight of shallots from 25 to 30 per cent. Yellow dwarf is hard to control under Louisiana conditions. The growing of disease-free seed stocks in isolated areas is helping to reduce the amount of yellow dwarf.

Bottom-Rot of Celery Caused by Corticium vagum B. & C. WEBER, GEORGE F. The disease, difficult to detect in fields, appears on the basal leaflets and succulent petioles of mature celery plants growing in dense stands on wet, highly organic soils under continued rather high humidity. Margins of leaf blades first show the disease, becoming wilted, blackened, and wet. The disease progresses toward the midribs and larger veins. It usually appears on young leaflets, spreading toward the center of the bunch but seldom into the heart of the plant. Loss of foliage is not excessive and early symptoms of the disease are not obvious, except in certain instances where the fungus spreading from the blade of the leaflet involves the secondary petioles and reaches the main petiole, causing collapse of the entire leaf. At the soil line near the base of the petioles or stalks, brown, circular to oval, slightly sunken lesions up to 1 in. or more in diameter may be observed on either outer or inner surface. The epidermis over these brown areas is intact and free from obstructions except for a barely discernible thin net of closely adhering, intertwined, brown, septate hyphae. These hyphae extend over the surrounding noncolored stalk tissue and frequently terminate in sclerotium-like tufts. The brownish luster, caused by the hyphae, of the surface of the petioles as observed in sunlight on a plant removed from the soil is intense at the soil line, fading to white several inches above. At this zone, the sexual stage of the fungus developed in a compact net of white superficial hyphae. Basidia and basidiospores were observed. On potato-dextrose agar the fungus produced characteristic mycelium and sclerotia.

Factors Affecting the Efficiency of Peanut Seed Treatments. WILSON, COYT. Increases in emergence of peanuts resulting from seed treatment with a dust disinfectant depend upon quality of seed, type and dosage of disinfectant, and storage conditions between time of treatment and time of planting. Increased emergence was greater on poor quality than on good quality seed. Average emergence over a 5-yr. period on untreated, machine-shelled seed was approximately 60 per cent. Emergence of treated, machine-shelled seed was approximately 80 per cent. Emergence of untreated, hand shelled seed was about 80 per cent. Treatment increased emergence to about 86 per cent. Significant increases in emergence were obtained from the following seed protectants, arranged in ascending order: Spergon (98 per cent tetrachloro-para-benzoquinone), Dow 9B (50 per cent zinc trichlorophenate), Phygon (98 per cent 2,3-dichloro-1, 4-naphthoquinone), Arasan (50 per cent tetramethyl thiuramdisulfide), 2 per cent Ceresan (2 per cent ethyl mercury chloride), and Ceresan M (7.7 per cent ethyl mercury p-toluene sulfonanilide). Dosage was more important with mercurials than with organic treatments. Increasing the dosage of Phygon from 3 to 6 oz. per 100 lb. of seed did not appreciably affect emergence, but increasing the dosage of Ceresan M from 1½ to 3 oz. per 100 lb. decreased emergence almost 20 per cent. When the dosage of Ceresan M was increased to 6 oz. per 100 lb. there was almost no emergence. Treatments were less effective in preventing deterioration of seed at summer temperatures than at winter temperatures.

REPORT OF THE SIXTH ANNUAL MEETING OF THE POTOMAC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The meeting was held in the auditorium of the U. S. Plant Industry Station, Beltsville, Maryland, February 23 and 24, 1949. Approximately 100 members were in attendance. Seventy members and guests attended the annual banquet at the conclusion of the first day's sessions, followed by an illustrated lecture on "Agricultural Potentialities of the American Tropics" by M. H. Langford. During the business session, members discussed their preferences as to time of meeting of the parent society.

Officers elected to serve during the ensuing year were: *President*, C. S. LEFEBVRE; *Vice-President*, C. E. COX; *Secretary-Treasurer*, J. B. DEMAREE; *Councilor*, PAUL R. MILLER.   J. B. DEMAREE, *Secretary-Treasurer*

ABSTRACTS OF PAPERS

Natural Control of the Destructive Sweetclover Weevil Sitona cylindricollis Fabr. by an Entomogenous Fungus Parasite. ALLISON, J. LEWIS. Recent findings indicate that a natural biological control has been effective in destroying the sweetclover weevil in certain endemic localities. An entomogenous fungus parasite has been discovered to be the causal agent in this control. The fungus has been identified as a member of the genus *Hirsutella* and represents a new species. No perfect or ascigerous spore stage of the fungus has been found in nature or induced under laboratory conditions. None of the entomogenous fungi have been heretofore reported as parasites of the sweetclover weevil. The fungus attacks only living weevils but does not produce its horn like clavae on which the conidia are borne until after the insects die and become mummified. The adult is the only stage in the normal cycle of the weevil that has been found parasitized. A feature in the life history of the weevil makes it especially vulnerable to attack by its fungus parasite. There is only one generation a year. The newly-developed adults appear during the summer but they rarely mate and oviposit until the following spring. As the fungus parasite is most destructive during late summer and early fall, it parasitizes many weevils prior to mating and subsequent oviposition. As in the case of all entomogenous fungi which have been reported as effective agents in biological control, this fungus is no exception in its pattern of cyclic development. The fungus parasitizes and destroys some weevils each year but does not become an effective agent in control until the sweetclover weevil population in a given area attains a peak. At this stage the fungus, which has been gradually building up, spreads rapidly attaining epidemic proportions and in a short time reduces the weevil population to a bare minimum. The cycle for both insect and fungus parasite must then start anew.

Chrysanthemum Stunt. BRIERLEY, PHILIP, and FLOYD F. SMITH. Chrysanthemum stunt, first recognized in 1945, became generally prevalent in greenhouse chrysanthemums in this country and Canada in 1946. Affected plants are smaller than normal, leaves are smaller and paler green, flowers smaller, and in some bronze- and pink-flowered varieties the color is bleached. Graft transmission was 100 per cent successful in 1947, but 55 per cent of the control plants also developed the disease. Transmission trials made between January and May 1948 resulted in expression of symptoms in only 5 of 50 grafts in August. When the test plants were flowered again in December 1948, stunt appeared in all of the 50 plants grafted with stunt scions, all of 48 inoculated by leaf rubbing, 10 of 35 inoculated by *Rhopalosiphum rufomaculatum* (Wilson), in low and perhaps nonsignificant proportions from transfer of 4 other aphid species, and in 6 of 100 noninoculated controls. The minimum observed period for expression of symptoms following grafting is about 3½ months; a more common interval is 7 to 10 months, or in the second flowering period after inoculation. Control practices indicated are selection of healthy plants during two successive flowering periods, control of aphids, and precautions against handling hazards.

Host-Parasite Interrelationships of Nematodes in Root Diseases. CHITWOOD, B. G. This is an extremely broad subject, brought forward with the idea that factors often not considered caused field observers and experimentalists to reach false conclusions. Briefly, the variables in nematode-plant interrelationships are as follows: (1) Inoculum: A. Nematode genus, species, variety or race, genotype. B. Quantity of inoculum. (2) Effluvium: A. Attractant. B. Repellant or Inhibitor (this is a chemical by-product of root growth). (3) Plant: A. Quality, *i.e.*, genus, species, variety. B. Quantity at a given time. (4) Conditioning factors, including climatological and soil conditions, their effects on plant quantity and effluvium and on the nematodes' reaction to these factors. (5) Hazards to nematode, such as environmental risks and natural enemies. (6) Invasion of plant: A. Enzymes (action on plant). B. Mechanical destruction. C. Plant response. (7) Parasite and host growth and nutrition: A. Ability of plant to feed nematode properly. B. Possible poisonous materials in plant. C. Materials withdrawn from plant by nematode, as to quantity and quality (possible differential absorption). D. Rate of reproduction of nematode (conditioning factors: food, temperature and heredity). (8) Subsequent invasions and subsequent nematode generations. (9) Secondary invaders. (10) Transmission of other organisms by nematodes. If any of the above ten variables is changed the entire interaction may be changed.

The Production of Internal Bark Necrosis of Apple in Sand and Soil Cultures. CLULO, GENEVIEVE. The disease was produced at the end of the first growing season by adding 64 or more p.p.m. manganese in the form of manganous sulfate to sand cultures. Two soils were used: one on which the disease always developed; the other from a location where Delicious had been grown for twenty years without becoming diseased. MnSO$_4$, H$_2$SO$_4$, or sulfur added to either soil increased the incidence and severity of the disease on bark formed the previous season, less intensely on the current season's bark. (NH$_4$)$_2$SO$_4$ and NaNO$_3$ added to the disease-producing soil increased the incidence and severity of the disease on the current season's bark with no apparent symptoms on the bark of the previous season. When Ca(OH)$_2$, CaCO$_3$, MgCO$_3$, MgO, or Na$_2$CO$_3$ was added to disease-producing soil in the greenhouse the development of the disease was prevented for the duration of the experiments (5 years). These alkaline reagents increased the soil pH to 6.3–7.5. Trees planted in the orchard in soil treated with Ca(OH)$_2$ have not become diseased during a 10 year period. Individual trees often vary widely in their ability to absorb Mn under identical conditions. A pronounced oedematous condition of the bark may accompany the typical pimply symptom and is usually associated with treatments which stimulate rapid intake of Mn.

An Undescribed Virus Disease of Honeysuckle. CORP, V. H. *Lonicera brachypoda* var. *folus aureo-reticulatis*, propagated for ornamental purposes, has been found to be *L. japonica* infected with a virus. This virus has been reported graft-transferable by Woods and DuBuy (Phytopath. 33: 637–655. 1943). Recently the virus has been graft-transferred four successive times without decrease in severity or other change in symptom pattern to the green seedlings of the yellow-veined parent and to *L. japonica* collected in the field. Within 15 to 20 days after grafting, the veins of leaves of the stock appear yellow. Symptoms vary from the original when the field-collected *L. japonica* clone is used as grafting stock. Yellowing appears along veins in young leaves and, as the leaf matures, spreads to interveinal areas resulting in a mottled appearance. There is no evidence that this virus is seed-transmitted or spread in the field other than vegetatively.

Botryosphaeria and Diplodia Associated with Cankers on Linden and Redbud. DAVIDSON, ROSS W., HORACE V. WESTER, and MARVIN E. FOWLER. Several strains of *Botryosphaeria ribis* and one of *Diplodia theobromae* were isolated from large conspicuous cankers on *Tilia neglecta* in the parks of Washington, D. C. These cultures were tested by inoculation into stems of several species of linden. *T. neglecta* was most susceptible to the fungi, as evidenced by the number of successful inoculations and the size of cankers formed. The tests indicated that *T. cordata, T. americana, T. dasystyla, T. platyphyllos,* and *T. europaea* were less susceptible to the fungi. The large perennial type of cankers on *T. neglecta*, from which the cultures were obtained, have not been reproduced in these inoculation tests. A common and destructive canker of *Cercis canadensis*, in the vicinity of Washington, D. C., is believed to be caused by *B. ribis*. Cultures of this fungus from cankers on redbud and on linden caused cankers when inoculated into stems of *C. canadensis* and *C. chinensis* although the latter host is not known to be susceptible to natural infection by *B. ribis*. Check inoculations with sterile culture media in small stem wounds resulted in no cankers on any of the hosts.

Resistance of Lycopersicon hirsutum × L. esculentum Hybrids to Infection with To-bacco Mosaic Virus by Handling and Pruning. DOOLITTLE, S. P., and W. S. PORTE. Nearly all plants of segregating progenies of mosaic-resistant *Lycopersicon hirsutum* × *L. esculentum* crosses develop mosaic symptoms when inoculated with a yellow strain of tobacco-mosaic virus by leaf rubbing with use of carborundum powder. Many such plants, however, resist infection by means comparable to those causing natural infection in field and greenhouse. When inoculated (without carborundum) by lightly rubbing one leaflet with the virus diluted 1–10, 1–50, 1–100, 1–1000, and 1–10,000, series of ten young Marglobe plants were uniformly infected. Identical tests with three hybrid lines showed an average infection of 84 per cent at 1–10, 50 per cent at 1–50, 26 per cent at 1–100, 25 per cent at 1–1000, and 10 per cent at 1–10,000. When five lots of ten plants of each hybrid line were handled as in transplanting after first handling a mosaic plant, the average infection for all lines was 9 per cent and for Marglobe 66 per cent. When ten plants had one leaf pruned with a knife first used to prune a mosaic plant, the average infection in fifty plants of each hybrid line was 5 per cent and for Mar-globe 40 per cent. When tops of ten plants were cut off with a knife carrying mosaic virus, forty plants of each hybrid line averaged 22 per cent infection and Marglobe 61 per cent.

Longevity of Some Pythium Species in Maize-Meal Agar Cultures. DRECHSLER, CHARLES. Many cultures of *Pythium* species grown in test-tubes on maize-meal agar containing some maize meal in suspension were successfully transferred 6¼ to 7 years after inoculation. On adding sterile water to the substratum, which had been in an air-dry, horny condition for about 4 years, some cultures of *P. ultimum* and *P. oli-gandrum* showed normal internal organization in fully 95 per cent of the very large number of oospores present. Survival in lesser ratios was noted among oospores of *P. debaryanum, P. irregulare, P. mamillatum, P. vexans, P. salpingophorum, P. disso-tocum, P. complens, P. acanthicum, P. periplocum, P. oedochilum, P. palingenes,* and *P. ostracodes.* On addition of sterile water 9⅞ years after their inoculation, cultures of *P. Violae, P. oligandrum, P. acanthicum, P. oedochilum,* and *P. palingenes* showed living oospores of correct internal structure present in the resoftened agar, and transfer to fresh substratum resulted in renewed growth of those species. Some transfers from old cultures were unsuccessful, although oospores of correct living structure were pres-ent. In these instances presumably all surviving oospores were in a dormant condi-tion, and would have required a period of after ripening before becoming capable of germination. The culture of *P. Violae* used was isolated in May 1938 from softened cortex of diseased pansy roots dug up in Washington, D. C.

Natural Spread of Phony Disease to Apricot and Plum. HUTCHINS, LEE M., and JOHN L. RUE. Natural spread of phony disease to seedling trees of two species of apricot and three species of plum was observed at Fort Valley, Georgia. The species affected and the severity of symptoms under the cultural conditions provided were as follows: symptoms pronounced in common apricot (*Prunus armeniaca*); less marked in *P. hortulana* (Hortulan plum) and *P. mexicana* (big tree plum); highly indefinite in *P. mume* (Japanese apricot) and *P. angustifolia* (chickasaw plum). Proof of infec-tion was established through indexing by root grafts to normal peach nursery trees. Where the root piece carried the virus, the index tree developed phony disease after an incubation period of 18 months. Infected trees of the latter two species were virtually symptomless carriers of the virus. The hazard of spread of phony disease from infected wild plums to near-by peach orchards is discussed.

Roadside Survey for Spot Anthracnose of Flowering Dogwood in Maryland, Vir-ginia, and West Virginia. JEHLE, R. A., and ANNA E. JENKINS. In reporting the existence of a spot anthracnose of flowering dogwood (*Cornus florida*) and describing its pathogen (*Elsinoë corni*), Jenkins and Bitancourt (1948) included one locality in Maryland (Beltsville) and one in Virginia (Norfolk) as the known distribution of the disease. A survey for this spot anthracnose conducted during summer and autumn of 1948 reveals that in Maryland it is widespread and often severe in the southern part and that its prevalence and severity gradually diminish to the northward until it seems to disappear just above the 39th parallel. It was not detected at all in northern Maryland. It was found in two localities in Sussex County, southern Delaware and in three localities in Accomack County, on the Virginia Eastern Shore. In northern Vir-ginia and northern West Virginia along route 50 from the District of Columbia to Allegheny Front no evidence of the disease was found.

Studies on the Overwintering of Monilinia fructicola in Peach Twig Cankers. KEPHART, JOYCE E., and JOHN C. DUNEGAN. As part of the studies on twig canker fun-

gus sporulation, tissue fragments of overwintered peach twig cankers from Michigan were cultured from January to April 1948, and similar work started on material from Virginia in January 1949. *Monilinia fructicola* was recovered from 71 per cent of the Michigan cankers and 30 per cent of the Virginia cankers. Culture plates from the Virginia cankers had a large number of saprophytic fungi whereas the plates from the Michigan material usually were pure cultures of *M. fructicola*.

Johnson Grass Smut in Relation to Sorghum Culture. LEUKEL, R. W., and JOHN H. MARTIN. Smut commonly infecting Johnson grass (*Sorghum halepense* (L) Pers.) was found to be identical with *Sphacelotheca holci* identified in 1934 by H. S. Jackson on sorghum in Venezuela. As previously noted, Johnson grass smut differs from *S. cruenta* in that its spores are larger, darker, more echinulate, and shorter-lived. It differs also in its limited host range and in its severe stunting effect upon susceptible plants. Infection may be induced by seed inoculation with chlamydospores, or by applying spores to freshly cut stubble. The mycelium was found to travel down into the crown, up into other shoots or culms, and into and along the rhizomes to infect other more distant shoots. It is not considered a serious menace to sorghum because of its short-lived spores.

Controlling Late Blight by Spraying Potato Cull Piles with Sprout Inhibitors. MARTH, P. C., and E. S. SCHULTZ. Waste potatoes that harbor the late-blight fungus and are deposited on cull piles are sources for initial late-blight infection. Cull piles also harbor potato insect pests. Potato tubers were sprayed with sprout inhibitors, or dormancy treatments, as they were deposited on cull piles, to determine the effect of such treatments on sprout inhibition. In exploratory tests during 1944 to 1947 in the Beltsville greenhouse, applications made with the methyl ester of naphthaleneacetic acid, with 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and with the methyl, ethyl, butyl, and isopropyl esters of this latter acid, were outstanding in preventing sprouting on Green Mountain, Katahdin, and Sebago. In 1947 at Presque Isle, Maine, potato cull piles were treated by spraying each layer of tubers with a 2500 p.p.m. concentration of 2,4-D (acid) and of 2,4,5-T (acid). Only 2,4,5-T prevented sprouting. In further field tests made in Maine in 1948 with the methyl ester of naphthaleneacetic acid and with the butyl and isopropyl esters of 2,4,5-T at a spray concentration of 2500 p.p.m., sprouting was prevented and sprout growth on sprouted tubers was inhibited.

Quarantine Aspects of Prunus Virus Control. McCUBBIN, W. A. Prunus virus control programs aim both to provide virus-free planting stock and to protect the resulting long-lived orchard from infection. Some control measures can be carried out well by the grower or nurseryman; others require special technical aid; and in a number of cases regulatory supervision is needed. Control methods include 1) bud-testing techniques; 2) marking and removal practices; 3) isolation arrangements; 4) vector suppression; and 5) treatments (when these are available). In all these activities regulatory powers are likely to be called on in varying degree and in combination with grower and technical efforts to secure program completeness, to provide official certification, to maintain isolation requirements, and to assist in vector suppression. Because of the nature of the problem, regulatory features must be undertaken largely by the State. Federal authority, effective in the foreign virus control field, is not adapted to deal with many phases of domestic control.

Variability in Monoconidial and Hyphal-Tip Isolates of Helminthosporium turcicum. ROBERT, ALICE L., and MERLE T. JENKINS. A wide range of virulence exists among monoconidial isolates of *Helminthosporium turcicum*. Pathogenicity of these isolates may change from one year to the next when grown on artificial media or when passed through a susceptible host. Twenty-five monoconidial isolates from each of two leaf lesions caused by two different monoconidial cultures differed pathogenically. Similar variation was obtained with ten hyphal-tip isolates from each of five single conidia. Some of the hyphal-tip isolates caused no infection. Growth of these isolates on potato-dextrose agar showed differences in cultural characters, rate of growth, and production of conidia. Of twelve non-spore-producing hyphal-tip cultures, ten produced no infection and two produced very slight infection.

Pulling vs. Spraying Potato Tops with Herbicides for Control of Virus Diseases. SCHULTZ, E. S. Pulling healthy potato tops August 1 in Aroostook County, Maine, before the plants are infested with viruliferous aphids, results in healthy seed potatoes. Recently potato tops were killed with herbicidal sprays to compare this method for control of virus diseases with the more laborious one of pulling the plants. In 1944 tests the tops were treated August 15 and the tubers harvested September 2. Pulling tops

resulted in 6 per cent leaf roll; Sinox spray, 14 per cent; and controls, 33 per cent. In 1946 tops were treated August 23 and tubers harvested September 6. Pulling tops resulted in 2 per cent mosaic; sulfuric acid spray, 2 per cent; Sinox spray, 4 per cent; and controls, 20 per cent. In 1947, tops pulled, sprayed with Sinox General, with Vine Kill, and controls, treated August 27 and harvested September 9, resulted in, respectively, 3, 4, 3, and 12 per cent mosaic. The results in 1944 indicate that the stalks remaining alive for two weeks after spraying with an herbicide transmitted some additional leaf roll to the tubers when aphids were numerous. In 1946 and 1947 the treated stalks alone did not transmit virus diseases when aphids were scarce. These results indicate that virus diseases can be controlled by killing the tops early in August with herbicides when the tubers are harvested a few days after the tops are killed.

Further Studies on Barley Powdery Mildew (Erysiphe graminis hordei). TAPKE, V. F. A previous investigator reported that, in greenhouse-grown barley plants, resistance to infection with powdery mildew varies directly with the age of the plant at the time of inoculation. The writer's results indicate that not necessarily the age, but rather the relative tenderness or toughness of the plant tissue as conditioned by environment, is the critical factor. At Beltsville, Maryland, five varieties of barley plants in the heading stage in the greenhouse proved highly susceptible when inoculated in December, January, and February, but infection progressively declined from March through the conclusion of the test in May. Evidently this was due to a gradual change from the lush, tender, susceptible tissue developed in the dim, short, cool days of December, January, and February to a tougher, less susceptible tissue developed in the longer and usually much brighter, warmer days accompanying the progress of the season during March, April, and May. This is in line with the writer's recently reported studies showing that the environmental conditions to which the plants are exposed before inoculation may materially influence their reaction to mildew after inoculation.

Growing Two Annual Greenhouse Crops Instead of One in Studies on Barley Smuts. TAPKE, V. F. Under the controlled conditions of greenhouse culture, exceedingly rapid progress can be made in studies on physiologic races of the covered and two loose smuts of barley, on varietal resistance to the various races of each smut, etc. The writer has developed a mass-production method of testing barleys for smut resistance whereby, in his allotted space, 61,568 plants are grown instead of the 9,190 plants that would be grown under methods commonly used in the past. Under the new intensive system, seed is germinated in small pans and seedlings are transplanted 1 in. apart in rows only 2 in. apart. The plants are lightly watered. This restricts growth, saves water and time spent in watering, and practically eliminates weeds and mildew. Studies have shown that in the greenhouse very small plants yield data as reliable as that from very large plants. Two crops are grown annually. The first is transplanted in the greenhouse September 10. Artificial light is applied 1 hr. daily, midnight to 1 A.M., from mid-October to heading in late November or early December. The second crop is transplanted in January. Under this system the field hazard of failure due to unfavorable conditions for infection is eliminated.

Studies on Selenium Therapy of Meadow Nematode-Infected Boxwood. TARJAN, A. C. Active adult meadow nematodes from infected boxwoods were immersed in solutions of 5 to 1000 p.p.m. of sodium selenate. None of the concentrations used had any noticeable effect on the nematodes. However, application of solutions of 25, 50, and 100 parts of sodium selenate per million of soil to the soil around roots of 12-yr.-old nursery-grown boxwoods, *Buxus sempervirens angustifolia* West and *B. sempervirens handsworthii* Dallimore, caused striking fluctuations in root nematode populations in contrast to the controls. Similar results were evidenced in populations of free-living nematodes in the soil around the roots of treated plants. A special sampling technique involving a 3-wk. immersion of nematized root samples in Baermann funnels was used in obtaining quantitative determinations of nematode populations in boxwood roots. Concentrations of 50 and 75 p.p.m. of sodium selenate powder were applied as a soil amendment around the roots of 13-yr.-old plants. Root samples were taken at bimonthly intervals for a period of 5 months. Resulting nematode counts for the selenium-treated plants were lower at the 1 per cent level of significance than nematode counts for the controls. Apparently selenium is ingested by the feeding nematodes and a toxic reaction results.

Following are titles of other papers presented at the meeting of the Potomac Division of which abstracts were not submitted for publication:

Rhizoctonia solani, a Destructive Pathogen of Alta Fescue, Smooth Brome Grass and Birdsfoot Trefoil. J. LEWIS ALLISON, HELEN S. SHERWIN, IAN FORBES, JR., and ROBERT E. WAGNER.

Cultural Characteristics of Several Species of Trametes. ROSS W. DAVIDSON and FRANCES LOMBARD.

Effective and Parasitic Strains of Rhizobium. S. W. ERDMAN.

An Undescribed Species of Periconia on Sorghum. C. S. LEFEBVRE, A. G. JOHNSON, and HELEN S. SHERWIN.

Agricultural Potentialities of the American Tropics. M. H. LANGFORD.

Incidence of Fusarium Infection as Affected by Root-Knot Nematodes. W. D. McCLELLAN and J. R. CHRISTIE.

A Review of 1946–48 Weather Conditions in Relation to Late Blight Phytophthora Infection. MURIEL O'BRIEN.

A New Disease of Pin Oak, Possibly Caused by the Nematode Hoplolaimus coronatus Cobb. R. M. VIGGARS and A. C. TARJAN.

Pathological Observations in the Low Countries. W. H. WHEELER.

PHYTOPATHOLOGICAL NOTES

Root Inoculation, a Method Insuring Uniform Rapid Symptom Development of Bacterial Ring Rot of Potato.— In greenhouse or field studies with bacterial ring rot of potato caused by *Corynebacterium sepedonicum* (Spieck. and Kotth.) Skapt. and Burkh., the need arises for a reliable, efficient method of inoculation. With previously employed methods, such as hypodermic injection in the sprouts, needle puncture in the eyes, or momentary dips in bacterial cultures, infection and resultant symptoms do not always occur. Frequently only one stem of a plant becomes infected while another from the same seed piece remains healthy; or infected plants may develop symptoms only unilaterally.

The fact that these methods afford entrance of the agent into only a few of the many vascular elements of the plant perhaps accounts for the irregular development of symptoms. Larson,[1] working with tomatoes, obtained ring-rot symptoms in plants transplanted to soil freshly inoculated with ring-rot organisms, indicating that invasion occurred through damaged roots. Tyner[2] demonstrated that potato plants could be infected readily by dipping wounded root tips in a bacterial suspension, although he obtained negative evidence of the occurrence of field spread by row irrigation water.

With a view to determining the degree of uniformity of infection and the rate of symptom development in root inoculated plants, 10 seed pieces of Bliss Triumph potatoes were sprouted and rooted in moist peat moss for 20 days at 20° C., at the end of which period 1- to 2-in. sprouts and 2- to 4-in. roots had formed. These plants were removed from the peat moss, the roots were washed in running water, and the majority of roots were cut with scissors ½ in. from their tips. Cut roots of 10 plants were dipped for 10 sec. in a water suspension of infected tissue expressed from the vascular ring of a ring-rot tuber. Two plants with cut roots, not so dipped, were retained as controls. Immediately following inoculation all seed pieces were planted in moist soil in 6-in. porous clay pots and placed at 22° C. Plants were observed daily in order to detect the first symptoms of ring rot.

Ring-rot symptoms developed rapidly and with unusual uniformity in 32 to 38 days following inoculation. The average number of days elapsing from inoculation to first expression of symptoms was only 35, which is a much shorter period than the 50 days at 22° C. air temperature or 44 days at 22° C. soil temperature, which were determined in other experiments to be required by plants hypodermically sprout-inoculated. Of greatest interest was the fact that all root-inoculated plants became infected and developed disease symptoms very rapidly and uniformly fol-

[1] Larson, R H. The ring-rot bacterium in relation to tomato and eggplant. **Jour. Agr. Res.** [U. S.] 67: 309–325. 1944.

[2] Tyner, L. E. Studies on ring-rot of potato caused by *Corynebacterium sepedonicum*. **Sci. Agr.** 27: 81–85. 1947.

lowing the earliest symptoms on the lower leaves. Control plants remained healthy. At harvest, 62 days after planting, all leaves of inoculated plants had developed typical ring-rot symptoms including severe wilt.

In a complementary experiment the quantitative aspect of root inoculation was investigated. Twenty seed pieces were sprouted and rooted in peat moss for 20 days at 20° C. After this period 15 to 20 roots had formed. Following removal from the moss the pieces were washed thoroughly in running tap water and divided into 6 lots of 4 each. All the roots in the first lot, ½ of the roots in the second, ¼ of the roots in the third, 2 roots in the fourth, and no roots in the fifth lot were severed ½ in. from their tips and dipped for 10 sec. in a water suspension of ring-rot diseased tuber tissue. A control lot in which roots were neither cut nor dipped was included. All seed pieces were planted in moist soil in 6-in. porous clay pots and retained at 22° C. All plants were observed daily for first ring-rot symptoms and were harvested 51 days after planting, when smears of all stems were made at a point 1 in. above the soil and were stained by the modified Gram stain method of Racicot, Savile, and Conners.[3]

Root inoculation resulted in 100 per cent infection followed by early uniform ring-rot symptoms in 26 days in all plants which had at least ¼ of their total number of roots cut and inoculated. In 3 of the 4 plants having only 2 roots cut and inoculated, symptom development was somewhat slower (29 days); also, one plant of this lot failed to develop symptoms although ring-rot organisms were detected in the stem. On the plants whose roots were not cut previous to dipping in the bacterial suspension, no symptoms were expressed during the 51-day period; however, 2 of the 4 plants were harboring moderate numbers of the causal organism in the aerial stems as shown by Gram-stain diagnosis. It is logical to assume that minor root damage occurred during the planting operation and provided entrance for small numbers of bacteria which were, however, too few to cause symptoms to be expressed. Plants in the control series remained healthy and carried no Gram-positive bacteria at harvest. Field studies at Lincoln in 1947 confirmed these greenhouse results.

To summarize, the root-inoculation method was found to be a most efficient technique for effecting ring-rot establishment and rapid uniform development of the disease in the potato plant. Also the number of infection courts, within limits, had little influence upon the rapidity or severity of ring-rot symptomatology since as few as 2 roots provided entrance for enough bacteria to produce disease symptoms of average severity.—
ARDEN F SHERF, Department of Plant Pathology, University of Nebraska, Lincoln, Nebraska.

[3] Racicot, H. N., D. B. O. Savile, and I. L. Conners. Bacterial wilt and rot of potatoes, some suggestions for its detection, identification, and control. Amer. Potato Jour. 15: 312–318. 1938.

Relative Effects of Septoria lycopersici and of Possible Gaseous Emanations from Ripe Fruit on Defoliation of Tomato.—It is a common observation that defoliation of tomatoes occurs more rapidly on plants bearing a heavy load of fruit than on plants with few fruits or in the vegetative condition. No entirely satisfactory explanation has been offered. Samson[1] has demonstrated, in sand culture, that available nitrogen may be a factor, although he could not detect significant differences between fertilizer treatments in the field. Skok[2] conducted limited experiments both in the greenhouse and in the field and concluded that "it is probable that gaseous emanations from ripe fruits present on the vines are a major contributing factor resulting in defoliation of tomato plants as grown under commercial field culture. On the basis of what is known about such emanations from ripening fruits of various kinds, and on additional experimental evidence, ethylene may be one of the principal compounds involved." Since

TABLE 1.—*Defoliation of tomato as affected by fruition and Septoria lycopersici*

	Treatment							
Determinations	Non-dusted				Dusted			
	Fruit added	Normal fruit	Picked pre ripe	Deflor- ated	Fruit added	Normal fruit	Picked pre-ripe	Deflor- ated
Av. length of branch; inches	44.8	52.1	48.9	63.1	48.3	48.1	43.8	58.2
Av. portion of branch defoliated; inches	35.4	42.4	39.1	41.1	29.2	29.7	27.4	28.0
Av. per cent defoliated	79.0	81.4	79.9	65.1	60.5	61.7	62.6	47.8
Av. no. leaves lost per branch	18.6	21.0	20.0	19.4	14.7	15.2	14.5	14.8

it had been possible to cause almost complete defoliation of seedling plants by inoculation with *Septoria lycopersici* Speg. in the greenhouse, without the presence of fruiting plants, it seemed desirable to devise a field experiment to check Skok's hypothesis.

In 1943 two adjacent plots were set with Indiana Baltimore, a variety highly susceptible to *Septoria lycopersici*. The plants were infected when set in the field, June 4, and weather conditions favored development of the disease throughout the season. Each plot consisted of 4 rows of 10 plants each. In each plot, (1) one row was kept completely sterile by removing all flower buds; (2) in one row the fruits were picked just as they began to turn pink; (3) in one row the fruits were allowed to ripen normally; and (4) in one row the fruits were allowed to develop normally but additional ripe fruits, equal to the normal load, were placed under the vines. As soon as the plants began to flower, one plot was kept dusted throughout

[1] Samson, R. W. Defoliation of deflorated, late-set and variously fertilized tomato plants. Purdue Univ. Agr. Exp. Sta. 53rd Ann. Rept. 1940: 41–42.
[2] Skok, John. Defoliation of tomato plant as a response to gaseous emanations from the fruit. Bot. Gaz. 104: 486–489. 1943.

the season with a copper fungicide, while the second plot was left untreated. No attempt was made to measure the quantity of dust, and applications were made coincident with rains or heavy dews, the purpose being to maintain dust coverage. On September 15, using 3 main branches of each plant as a sample, the average length of branch, the average length and percentage defoliated, and the average number of leaves defoliated per branch were recorded for both the dusted and the non-dusted plot. These data are recorded in table 1.

Statistical treatment of the data summarized in table 1 shows a highly significant difference in defoliation between dusted and non-dusted plants with no significant difference in defoliation between all other treatments. The percentage of defoliation was relatively constant in each plot, irrespective of treatment except that on a percentage basis, deflorated plants defoliated less. However, on the basis of actual leaf drop the deflorated plants did not differ from plants with fruit. Dusted plots retained about 20 per cent more foliage than non-dusted plots. This experiment demonstrated that under epidemic conditions, *Septoria lycopersici* is a major cause of defoliation.

It is recognized that the physiological condition of the plant may influence the extent of defoliation but the importance of gaseous emanations by the fruit as a major cause of defoliation was not demonstrated in this experiment.—GEORGE B. CUMMINS, Department of Botany and Plant Pathology, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

An Epizootic of Phyllocoptruta oleivora (Ashm.) on Citrus in Florida.—One of the most serious insect pests of citrus in Florida is the rust mite, *Phyllocoptruta oleivora* (Ashm), which inflicts injury to the epidermal cells of the fruit and gives the rind of the fruit a brown, rusty appearance. This mite usually reaches the point of maximum infestation during late June and early July, shortly after the beginning of the rainy season. At such times, rust mite populations may be so high that mites will be present on virtually every fruit and leaf. Single fruits may be so heavily infested that the mites plus their cast skins give the fruit a dusty appearance. Yothers and Mason[1] reported that following such a period of maximum infestation on unsprayed trees the mites disappeared very rapidly, and that by the middle or end of September a diligent search was required in order to find even single specimens.

During October, 1948, an epizootic of rust mites occurred at Lucerne Park, Florida, in an unsprayed control plot consisting of 30 grapefruit trees. The rust mite population remained stable and at high levels through July and August. In September the mites began to disappear, and in October the epizootic was at its height. Figure 1 shows that be-

[1] Yothers, W. W., and A. C. Mason. The citrus rust mite and its control. U. S. Dept. Agr. Tech. Bul. 176. 1930.

tween August and October the percentage of fruit infested decreased from
98 to 20.   In November there was a further decrease in the percentage of
fruit infested.

Examination of the fruit showed that normal or healthy, as well as
dead, rust mites were present.   A high percentage of the living mites were
dark yellow to tan, rather than the light lemon-yellow typical of healthy
rust mites.   These off-color mites generally were sluggish in their move-
ments.   The dead rust mites were tan to light brown.   The appearance of
the abnormal mites corresponded with the descriptions by Speare and
Yothers.[2]

Rust mites which were alive but definitely abnormal in color and slug-
gish in their movements were mounted on slides in lactophenol and studied
under the compound microscope.   These rust mites were partially or com-
pletely filled with endoparasitic hyphal bodies.   The means of infection is
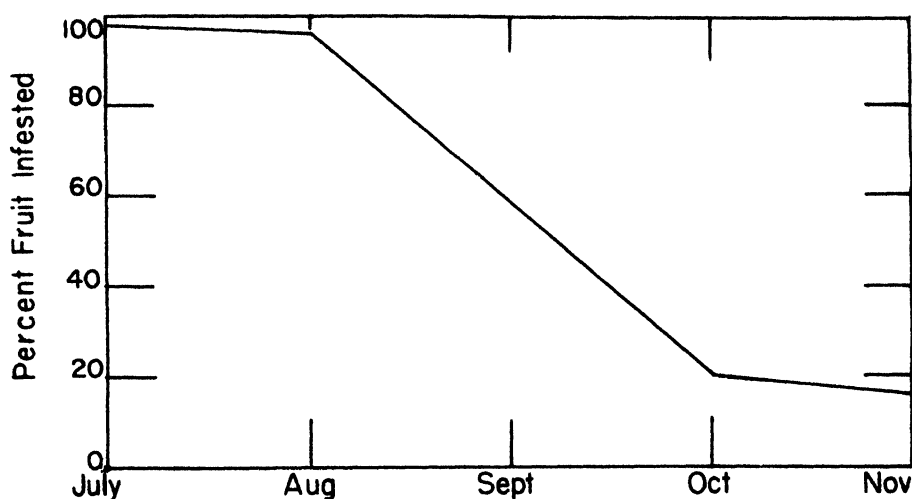
FIG. 1.   The percentage of citrus infested with rust mites in an unsprayed control
plot.   Lucerne Park, Florida.

unknown.   No hyphal bodies were found in normal rust mites of bright
lemon-yellow color.

As yet the identity of the endoparasitic hyphal bodies in living mites
has not been determined, but one fungus consistently associated with dead
mites has been tentatively identified as a species of *Hirsutella* Pat.   In
mites which were dead, mycelia were found growing from the interior
through both the anterior and posterior portions of the mites.   In some
specimens, which apparently had been dead for a long time, lateral emer-
gence of mycelia was also observed.   However, anterior and posterior
emergence of 1–4 mycelial strands was typical.   Under a hand lens, these
mycelia appeared as silver threads.   Throughout October and November
the fungus fruited abundantly.   At regular intervals along the mycelium,

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[2] Speare, A. T., and W. W. Yothers.   Is there an entomogenous fungus attacking
the citrus rust mite in Florida?   Science 60: 41–42.   1924.

flask-shaped phialides were produced which were terminated by one or two long, filiform sterigmata, each bearing one spore. It remains to be proved whether or not this fungus actually originated from the endoparasitic hyphal bodies found in the living mites.

Because spore production was meager in his specimens, Speare[3] believed that the fungus spreads from mite to mite by means of the external mycelia. If *Hirsutella* sp. originates from the endoparasitic hyphal bodies, then infection of the rust mites is probably by other means.—F. E. FISHER, J. T. GRIFFITHS, JR., and W. L. THOMPSON, University of Florida, Citrus Experiment Station, Lake Alfred, Florida.

Some Studies on Downy Mildew of Millet.—Difficulties encountered in testing breeding materials of millets for resistance to downy mildew (*Sclerospora graminicola* (Sacc.) Schroet.) necessitated a series of experimental inoculations at Peiping, China. Three methods were tested in the greenhouse in 1947: a vacuum method by which surface sterilized seeds of millet were placed in a suspension of oospores of the fungus for 15 minutes and subjected to partial vacuum, a heavy spore load method by which moistened millet seeds were thoroughly covered with oospores of the fungus, and a dehulling method by which oospores of the fungus were mixed with the millet seeds after the hulls were removed. With the vacuum method, 19 per cent of the plants were infected; with the heavy spore load, 55 per cent. The dehulling method was not a valid test because other pathogens attacked the seed before it germinated. There was 2 per cent downy mildew infection in control plants that had not been inoculated.

Two other methods, both unsatisfactory, were tried: application of oospores to the plumule and root of germinated seeds during the first five days after germination, and planting in soil infested with oospores. There was only 8 per cent infection when the inoculation was made 1 day after germination as compared with 15 per cent infection when inoculation preceded germination of the seed. Within 2 days after germination the plumule and root were longer than 3 mm., and inoculum placed there then or later did not cause infection. When seed was placed in infested soil one day after seed germination, only 7 per cent of the plants were infected as compared with 14 per cent infected when non-germinated seeds were planted in infested soil. Controls for these two types of inoculation had no downy mildew.

Naturally infested soil serves as a source of inoculum, but the infection resulting may vary somewhat according to date of planting. Surface disinfected seeds were planted in infested soil taken from a field in which there had been about 20 per cent downy mildew on the millet. There was 7 per cent infection in the planting of December 1 and 6 per cent in that of January 12; but 12 per cent in the February 25 planting and 29 per cent in that of March 31. All controls remained free of disease.

[3] A. T. Speare. Unpublished manuscript.

Temperature affects the development of downy mildew. Millet seeds were inoculated with a heavy load of oospores and were germinated for two days at 16°, 20°, 24°, 28°, 32°, or 36° C., after which they were planted in soil in pots and placed in greenhouses at 25°–28° C. or at 20°–24° C. At the higher greenhouse temperature the plants headed within 63 days and downy mildew infection was determined then; but 97 days were required at the lower greenhouse temperature. A range of temperature between 24° and 32° C. during the first 2 days was most conducive to development of mildew even though the temperature might fall to 20°–24° C. after the first two days (Table 1). When the temperature was as low as 16° C. during the first two days, not more than 9–11 per cent of the plants were infected.

It has been customary to determine susceptibility of millet varieties by counting the whitened or shredded plants and plants with leafy ears at heading time. It is possible, however, to determine infection earlier by

TABLE 1.—*The development of downy mildew in millet inoculated with oospores and germinated at various temperatures for 2 days before transfer to greenhouses*

Temperature during first 2 days after inoculation	Percentage of infection developed during	
	97 days at 20°–24° C.	63 days at 25°–28° C.
16° C.	9	11
20° C.	16	19
24° C.	40	28
28° C.	34	43
32° C.	60	22
36° C.	33	16

inducing conidial sporulation on younger plants. Conidia form readily on the dorsal leaf surfaces if infected plants are placed in a saturated atmosphere at approximately 24° C. for about 12 hours or overnight. The percentages of infection determined by this method for seedlings of various ages were as follows: 20 per cent at 1-leaf stage, 62 per cent at 2-leaf stage, 17 per cent at 4-leaf stage, and 5 per cent at 7-leaf stage. Obviously, the method should be used when seedlings are in the 2-leaf stage of development. When this method of determining infection was compared with the former practice of determining infection on mature plants, a correlation coefficient of 0.9 was observed in 35 experiments. Inducing the formation of conidia makes possible a considerable saving in time and a testing in the greenhouse during the winter.—MU-HWA PU and TZAN-MING SZU, Peiping Agricultural Experiment Station, National Agricultural Research Bureau, Peiping, China.

Reactions of Wheat Varieties to Stripe Rust, Leaf Rust, and Stem Rust In Peiping, China.—As preliminary to the scientific breeding of rust-resistant wheats for northern China, 787 domestic varieties of wheat collected

from 22 provinces of China and 656 foreign varieties were tested for their reactions to stripe, leaf, and stem rusts in Peiping in 1947–1948. The domestic collections included 651 farmer's varieties and 136 improved strains from various agricultural experiment stations and colleges. The foreign varieties included common and club wheats, durums, and emmers, and were introduced from the United States, various European countries, or Japan.

Many of the varieties were tested in the greenhouse and all were tested in field nurseries at Peiping. Inoculum of the three rusts, stripe rust (*Puccinia glumarum* (Schmidt) Erikss. and Henn.), leaf rust (*P. rubigovera tritici* Erikss. and Henn.), and stem rust (*P. graminis tritici* Erikss. and Henn.), was collected in the vicinity of Peiping in 1947. The physiological races comprising the inoculum were not identified, but according to unpublished data of H. R. Wang, race 123 of leaf rust and race 17 of stem rust are prevalent in that area. The most prevalent races of stripe rust are not yet known. Severe epidemics of the three rusts were induced in the nurseries by inoculating susceptible border rows with spore suspension by means of a hypodermic syringe. The fields were irrigated several times during the growing season to facilitate the spread of rust infection from border rows to the experimental varieties.

Most of the domestic Chinese wheats are susceptible to the three rusts. Of the 651 winter and spring varieties obtained from farmers, only four (1 from Hopei, 3 from Shantung) were resistant to stripe rust, only five (1 from Hopei, 2 from Shantung, 1 from Kansu, 1 from Kiangsu) were resistant to leaf rust, and only three (1 from Shantung, 1 from Kansu, 1 from Chahar)were resistant to both stripe and leaf rust. Not one was resistant to stem rust. Among the 136 improved Chinese varieties, 103 were highly susceptible to all three rusts, 13 were resistant to stripe rust, 14 were resistant to leaf rust, and 1 was resistant to stem rust. Four of the improved varieties, although susceptible in the seedling stage to stem rust, were resistant to all three rusts in the field. They were hybrids (No. 889, 918, 920, and 923) produced from a cross (made at the National Agricultural Research Bureau in Peiping) between a Chinese wheat and Percival's *Triticum albidum.*

The Chinese 166 wheat, which other workers have found resistant to many European races of stripe rust, was susceptible to stripe rust at Peiping.

Among the foreign introductions, numerous varieties of winter wheat were highly resistant to the three rusts. Resistant winter wheats from the United States were Cheyenne (C. I. 8885), Oro (C. I. 8220), Ioturk (C. I. 11388), Kanred (C. I. 5146), Poole or California Red (C. I. 3488), Turkey (C. I. 1558), Comanche (C. I. 11673), Tenmarq (C. I. 6936), and several hybrids from Kansas. Knoppies wheat from South Africa, Buisson from France, Almond from Sweden, Weizen Montcalm 245 from Switzerland, and Agr. No. 8 from Japan were highly resistant. Three winter wheats from Yenching University but of unknown origin also were highly resistant.

Hybrids originating from such crosses as Hope × Turkey, Comanche × Mediterranean-Hope, Oro × Mediterranean-Hope, Thorne × Mediterranean-Hope, or Mediterranean-Hope × Nebred were very susceptible to stem rust, both in the greenhouse and in the field.

Many of the spring wheats among the foreign introductions also were resistant to the three rusts at Peiping. Among them were Thatcher (C. I. 10003), Pilot (C. I. 11945), Merit (C. I. 12036), Marquillo (C. I. 6887), Marvel (C. I. 8876), Newthatch (C. I. 12328), Federation (C. I. 4734), Regent (C. I. 11869), and several hybrids from Minnesota. Several hybrids with Ceres-Hope-Florence × H44-Thatcher parentage were susceptible in the seedling stage to stem rust, but were moderately resistant in the field.

The variety Hope (C. I. 8178), which has long been highly resistant to or almost immune from many of the common races of stem rust in North America, was susceptible in the seedling stage and moderately susceptible in the adult stage to stem rust at Peiping. It was practically immune from stripe rust and highly resistant to leaf rust.

Triticum monococcum L., *T. Timopheevi* Zhuk., two varieties of *T. durum* Desf. (Acme, C. I. 5284, and Golden Ball, C. I. 6227), and one variety of *T. dicoccum* Sch. (Vernal, C. I. 3686) were highly resistant to or immune from the three rusts. Many varieties of *T. durum, T. dicoccum, T. spelta* L., *T. persicum, T. pyramidale* Mihi, *T. compactum* Host., and *T. macha* were resistant to the stripe and leaf rusts but were susceptible to stem rust. *T. sphaerococcum* Mihi and *T. orientale* Mihi were resistant only to stripe rust; and *T. turgidum* L. was resistant to none of the three rusts.

There seems to be little chance of obtaining the character of rust resistance by selection within the native wheat varieties of China. A breeding program based on the hybridization of Chinese wheats with wheats of foreign origin offers the best promise for securing rust-resistant varieties. The foreign wheats themselves are not entirely satisfactory because they generally mature 7 to 20 days later than Chinese wheats. In the winter wheat area of China, where stem rust usually is less severe than either stripe rust or leaf rust, it may be possible to use breeding materials that have resistance to the last two rusts. In the spring wheat area, where race 17 of stem rust is prevalent, some of the highly resistant Minnesota varieties may be used as parental material.—MU-HWA PU and OU-YANG YAO, Peiping Agricultural Experiment Station, National Agricultural Research Bureau, Peiping, China.

ANNOUNCEMENT

ANNUAL MEETING—1949

The forty-first annual meeting in New York City is tentatively scheduled for December 28, 29, and 30, 1949. To meet program publication deadlines set by the AAAS, the Secretary must have by October 5, *TITLES* of papers to be presented. No titles will be included in the AAAS general program if received by the Secretary of the APS after October 5. *ABSTRACTS* will be accepted by the Secretary until October 15.

The Abstract Committee may reject abstracts even though titles have been forwarded to the AAAS for inclusion in the general program. To avoid embarrassment authors are urged to make certain that the material to be presented meets the requirements of the Society and to give careful attention to the preparation of abstracts.

Projection costs will amount to about $25.00 per session for one lantern and operator. If two lanterns are required in a session it will cost $10.00 more. At this rate our projection costs are estimated at $425.00 if we have one lantern per paper reading session. The Society can save at least $170.00 by using slides of one size only. It seems that kodachrome is the preferable size because of the wide use of color. In planning and preparing material for presentation, authors are earnestly solicited to use only 2 × 2 slides, and thus to help keep down costs of the meeting. The Secretary of the AAAS is attempting to work out a plan by which a part of the costs for projection can be rebated by the AAAS to the participating societies. The amount of the rebate will be based upon membership, attendance, and registration with the AAAS.

Final arrangements for joint sessions with other societies, symposia, etc., must be completed by September 15, 1949, in order that meeting room assignments can be made; meeting rooms will be at a premium and it will be very difficult to change or increase the requirements after assignments have been made. Please let the Secretary know of your needs as soon as possible.

CURTIS MAY, *Secretary*
Plant Industry Station,
Beltsville, Maryland

A NEW VIRUS DISEASE OF LADINO CLOVER[1]

K. W. KREITLOW AND W. C. PRICE[2]

INTRODUCTION

A disorder of the Ladino variety of clover (*Trifolium repens* L.), characterized by chlorotic, yellow mottling of leaves, occurs extensively in the Northeastern United States (11). Severely affected plants frequently are stunted and bear distorted leaves. In the field, diseased plants occur individually or in spots one to several feet in diameter.

Plants of Ladino clover affected by the disease have been observed in central Pennsylvania since 1943, but some growers claim to have noted the disorder earlier. Diseased Ladino clover has either been seen in or received from the following States: Maine, Massachusetts, Vermont, Connecticut, Rhode Island, New Jersey, New York, Pennsylvania, Indiana, and Oregon.[3] More widespread culture of Ladino clover in recent years has undoubtedly increased the prevalence of the disease since it appears to be spreading and becoming more noticeable.

While no accurate estimates are available concerning the extent of damage caused by the disease, field observations indicate that it probably reduces yields by stunting the plants. Greenhouse observations show that affected plants are weakened and suggest that they are consequently more susceptible to adverse conditions such as drought and winter-injury.

The disease was readily reproduced by rubbing juice from infected plants onto leaves of healthy Ladino clover. Typical symptoms developed in from 12 to 21 days (Fig. 1, B). The causal agent is a virus, and these studies on its properties and on transmission demonstrate that the virus is a strain of alfalfa mosaic virus different from any previously described. The disease in Ladino clover is referred to as yellow patch and the virus is given the trinomial *Marmor medicaginis* H. var. *Ladino* n. var.

LITERATURE

Viruses infecting *Trifolium repens* L. in the field have been described by several authors. A number of additional viruses have been artificially transmitted to white clover. Zaumeyer and Wade (20, 21, 22) described a virus disease of *T. repens* that caused both systemic and local infection

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when transmitted to *Phaseolus vulgaris* L. Pierce (14) recovered from white clover a virus that infected *P. vulgaris* and several other species of legumes, but failed to infect soybean or tobacco. In a later test, Pierce (15) identified white clover virus 1 in five collections of diseased *T. repens*. Valleau (18) reported a virus disease of white clover, characterized by
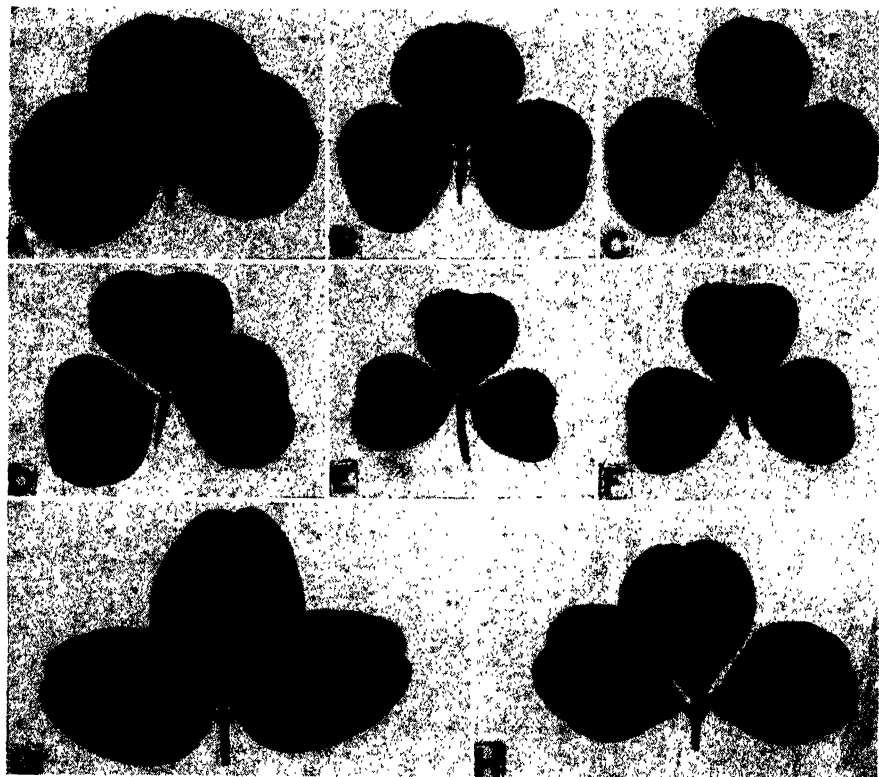
FIG. 1. Symptoms of yellow patch and white clover mosaic viruses in several species of *Trifolium*. Ladino clover: A. Healthy leaf. B. Leaf with yellow patch. C. Leaf with white clover mosaic. *T. incarnatum:* D. Healthy leaf. E. Leaf with yellow patch. F. Leaf with white clover mosaic virus. *T. pratense:* G. Healthy leaf. H. Leaf with yellow patch.

yellowish patches on leaves. The virus was transmitted mechanically to bean and tobacco. E. M. Johnson (10) isolated the same or a similar virus from *T. repens* which he transmitted to several hosts including tobacco. The virus produced local lesions on bean but failed to infect alfalfa, white clover, zinnia, or pepper. F. Johnson (8, 9) showed that a disease of white clover previously regarded as caused by a single virus, classified by Weiss (19) as Trifolium virus 1, was caused in reality by a mixture of two viruses. The viruses could be separated because dodder picked up and transmitted only one of the constituents and *Vigna sinensis* (Torner) Savi. was susceptible only to the other.

MATERIALS AND METHODS

Most of the virus material used in the tests was obtained from naturally infected plants of Ladino clover collected in the vicinity of State College, Pennsylvania. Seedlings of Ladino clover and other hosts tested were grown during the winter in flats or pots of steamed soil in a greenhouse that was fumigated regularly.

Healthy seedlings were inoculated by rubbing a square of sterile gauze dipped in juice of diseased plants over leaves previously dusted with carborundum powder. The leaves were then sprinkled with tap water and the plants were incubated in a greenhouse at 18–20° C. Controls were inoculated with juice from healthy plants.

For comparative purposes, most hosts tested were also inoculated with a white clover mosaic virus previously used by Atwood and Kreitlow (2) and maintained in a greenhouse in plants of Ladino clover. This virus produced symptoms resembling those produced by the white clover mosaic virus described by Johnson (8, 9).

SYMPTOMS

Symptoms in plants of Ladino clover inoculated in the greenhouse with yellow patch virus varied, depending upon susceptibility of the individual plant and on environmental conditions prevailing during the incubation period. In mildly affected plants, earliest symptoms developed in from 12 to 21 days and consisted of clearing of veins and mottling in newly developed leaves. In older leaves, the mottling gradually gave place to a condition in which yellowish areas appeared between veins and toward the margins of the leaf. In some cases, only a few small yellow areas ultimately developed in affected leaves and the plants usually showed no other visible damage.

In plants severely affected by yellow patch, earliest symptoms appeared in from 12 to 15 days. New leaves that unfolded were chlorotic and distorted. Later, green areas of unaffected leaf tissue delimited the somewhat angular, yellow patches characteristic of the disease. Necrotic areas occasionally developed in leaves of plants severely attacked. Stolon internodes frequently were greatly shortened and leaf petioles were stunted. Severely affected plants grew slowly, were weakened, and frequently failed to survive short periods of high temperature or excessive drying.

Symptoms in most affected plants were masked at high temperature. They developed most strikingly at 18–24° C. They tended to disappear during long periods of cloudy weather.

HOST RANGE

A number of species of plants were tested for susceptibility to yellow patch virus. Results of these tests are summarized in table 1. Symptoms produced by the virus in several hosts are illustrated in figures 1 and 2.

TABLE 1.—Comparative host range of yellow patch virus and white clover mosaic virus

Family and species	Number of plants infected[a]		Symptoms[b] of	
	Yellow patch virus	White clover virus	Yellow patch virus	White clover virus
Amaranthaceae				
Celosia argentea L. var. cristata Kuntze	2/2		L—SP—CL	
Apocynaceae				
Vinca rosea L.	10/10[c]		S—VC, SP—CL	
Balsaminaceae				
Impatiens holstii Engler and Warb.	2/2		S—SP—CL	
Chenopodiaceae				
Beta vulgaris L.	0/10	0/10	None	None
Spinacea oleracea L.	0/10	0/10	do	do
Compositae				
Callistephus chinensis Nees.	0/10	0/10	do	do
Lactuca sativa L.	0/10	0/10	do	do
Zinnia elegans Jacq.	9/10[c]	0/10	S—ST—C—CL	do
Cucurbitaceae				
Cucumis sativus L.	0/10	0/10	None	do
Leguminosae				
Glycine max (L.) Merr.	9/10[c]	0/10	S—ST—V, C, P—R	do
Lathyrus odoratus L.	5/8[d]	7/8	S—W—C—CL or L—SP—CL, N	S—M, VC
Lotus corniculatus L.	0/10	0/10	None	None
Lupinus sp.	0/2		do	
Medicago arabica (L.) All.	3/10[c]	0/10	S—ST—M, C—CL	S—M, VC
M. hispida Gaertn.	1/10[f]	2/10	S—M, VC—CL	S—VC
M. lupulina L.	0/3	0/3	None	None
M. obscura Retz.	3/3[c]	3/3	S—M, VC, C—CL	S—VC, C
M. orbicularis (L.) All.	1/2[c]		S—ST—M, VC, D—CL	
M. sativa L.				
Atlantic	0/10	0/10	None	None
Buffalo	0/10	0/10	do	do
Grimm	0/30	0/30	do	do
Ranger	0/10	0/10	do	do
Melilotus alba Desr.	8/10[d]	6/10	S—W—M, C—CL	S—M, VC, C
M. indica (L.) All.	6/10[c]	2/10	S—ST—M, VC, C—CL	S—M, VC, C
M. officinalis (L.) Lam.	2/3[d]	2/3	S—M—CL	S—M, VC
M. suaveolens Ledeb.	10/10[d]	8/10	S—K—M, VC, C—CL	S—ST—VC, C
Phaseolus lunatus L.	8/8[c]	0/8	S—M—CL	None

Family and species	Number of plants infected[a]		Symptoms[b] of	
	Yellow patch virus	White clover virus	Yellow patch virus	White clover virus
Phaseolus vulgaris L.				
Sure Crop Wax	18/19	0/17		
Northrup King Tendergreen	10/10	0/10		
Plentiful	8/8	0/8	L—SP—R	None
Burpee Stringless greenpod	9/9	0/9		
Woodruff Full Measure	9/9	0/9		
Bountiful	10/10	0/10		
Pisum sativum L.				
Marvel	24/26c	12/12		
Potlatch	25/28	11/14	S—ST—M, VC, C—CL	S—M, VC, C
Excelsior	22/26	13/14		
Blue Bantam	25/30	10/15		
Trifolium alexandrinum L.	2,10d	6/10	S—ST—M, VC—CL	S—VC, C
T. dubium Sibth.	4/10d	1/10	S—M, VC—CL	S—VC, C
T. fragiferum L.	6/10c	0/10	S—M, VC, C—CL	None
T. glomeratum L.	2,10d	1/10	S—M, VC, D—CL	S—M, VC, C
T. hirtum All.	7,10e	4/10	S—ST—C, D—N	S—ST—VC
T. hybridum L.	9/10e	5/10	S—ST—M, VC, C, D—CL	S—VC, C
T. incarnatum L.	10/10c	6/10	S—ST—M, V, C—B	S—VC, C
T. pratense L.	+c	+	S—ST—M, D—CL	S—M, VC, C
T. procumbens L.	0/10	0/10	None	None
T. repens L. (Ladino)	+c	+	S—ST—M, VC, D—CL	S—M, VC, C
T. resupinatum L.	10/10c	9/10	S—ST—M, VC, C, D—CL	S—ST—M, VC
T. subterraneum L.	9/10e	5/10	S—ST—M, VC—CL	S—ST—VC, C
Vigna sinensis (Torner) Savi.	5?/6d	0/6	L—V, SP—N, R	None
Scrophulariaceae				
Antirrhinum majus L.	7/10c	0/10	S—ST—VC, D—CL	None
Solanaceae				
Capsicum frutescens L.	9/10e	0/10	S—M, D—CL	None
Lycopersicon esculentum Mill.	0/10	0/10	None	None
Nicotiana glutinosa L.	2/2e		S—ST—M—CL or L—SP—CL	None
N. rustica L.	2/2c	0/10	S—ST—M, SP—CL	None
N. tabacum L.	10/10e	0/10	S—ST—M, P, D—CL	None
Petunia hybrida Vilm.	10/10e	0/10	S—ST—M, VC, C, D—CL	None

a The numerator indicates number of plants infected; the denominator, number of plants inoculated.
b Symptoms are designated by symbols:
Infection was local (L) or systemic (S).
Plants were stunted (ST), wilted (W), or killed (K) by the virus.
Leaf symptoms were mottling (M), vein discoloration (V), vein clearing (VC), downward curling (C), puckering (P), distortion (D), or spotting (SP).
c Tissues affected were chlorotic (CL) or necrotic (N); red (R) or brown (B).
d The virus was recovered.
d The virus could not be recovered.
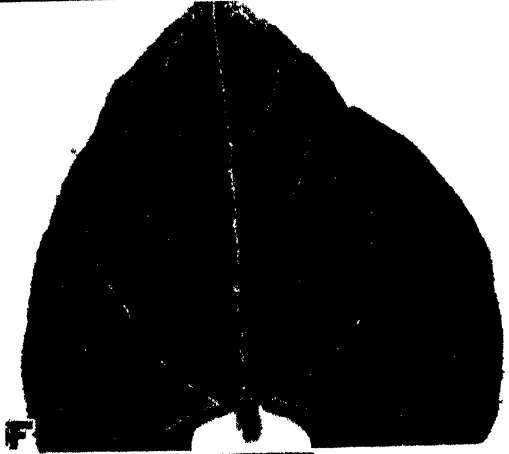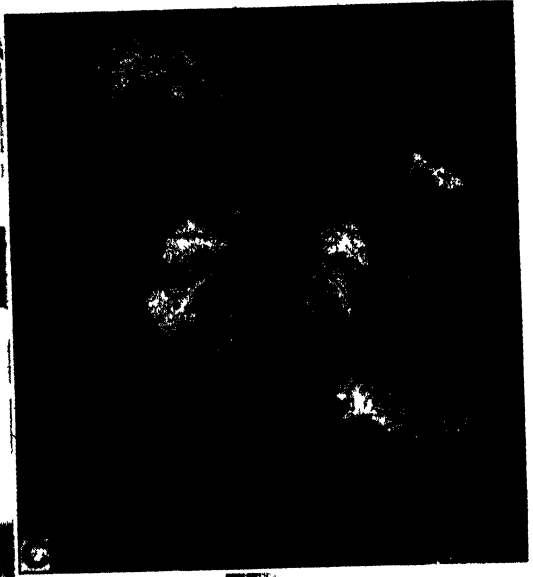
Yellow patch virus has a much broader host range than white clover mosaic virus. In common hosts, symptoms were usually distinctive. In most cases, symptoms produced by white clover mosaic virus were less severe than those produced by the yellow patch virus.

Symptoms produced by yellow patch virus were milder in some hosts than in others. Infected leaves of *Zinnia elegans* Jacq. and *Antirrhinum majus* L. developed a greenish-yellow chlorosis instead of the bright yellow chlorosis observed in other hosts. Leaves of zinnia curled downward only slightly and leaves of snapdragon twisted instead of curling downward.

Infected plants of *Glycine max* (L.) Merr. developed a deep green color that was maintained long after control plants matured and turned yellow.

Inoculated leaves of peas infected with yellow patch virus died prematurely. Plants with severe symptoms had greatly shortened internodes and produced leaves in tightly bunched clusters.

Symptoms of yellow patch virus in Turkish tobacco were similar to those caused by a strain of alfalfa mosaic virus isolated some years ago from a yellow spot that developed on a tobacco leaf infected with the type strain. Symptoms caused by the mutant strain are illustrated in figure 2, C.

Local lesions that developed on sweet pea were parchment-like and measured 3–5 mm. in diameter. They eventually coalesced so that infected leaves turned yellow, wilted, and dropped. Later, the terminal bud wilted and the plant died. When lateral branches developed before the plant died, they bore leaves that were small and curled.

The fact that yellow patch virus produced local lesions on bean afforded a convenient method of testing for the presence of virus in other hosts. Juice from leaves of hosts with symptoms of infection, or from hosts suspected of harboring the virus in a latent state, was used to subinoculate leaves of bean seedlings of the variety Bountiful. When local lesions measuring 1–2 mm. in diameter developed within 48–72 hours, the source host tested was considered infected with the yellow patch virus whether symptoms were evident or not.

Although several varieties of alfalfa (*Medicago sativa* L.) were inoculated, no infection was obtained in this host. No symptoms resulted from the inoculation, and virus could not be recovered by rubbing juice of inoculated alfalfa onto leaves of bean seedlings at frequent intervals following inoculation. Other species of *Medicago* varied in susceptibility to infection. Both *M. arabica* (L.) All. and *M. obscura* Retz. were susceptible

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Fig. 2. Symptoms of yellow patch virus produced in different hosts. A. (Right) Severe stunting in petunia (healthy plant at left). B. (Right) Stunting and curling of leaves in soybean (healthy plant at left). C. Chlorotic mottling in Turkish tobacco produced by a mutant isolated from the type strain of alfalfa mosaic virus. The symptoms are identical with those produced by the yellow patch virus. D. Leaf of healthy pepper plant. E. Chlorosis in pepper leaf infected with yellow patch virus. F. Leaf of healthy Bountiful bean. G. Local lesions produced in Bountiful bean, by yellow patch virus.

while *M. lupulina* L. and *M. hispida* Gaertn. resisted infection. *M. obscura* also was readily infected by white clover mosaic virus. Several species developed virus-like symptoms following inoculation with the yellow patch virus but local lesions failed to appear when juice from these plants was sub-inoculated to leaves of bean seedlings.

In general, most species of Leguminosae tested were susceptible to yellow patch virus. Other susceptible species were found in the Compositae, Scrophulariaceae, Solanaceae, Apocynaceae, Amaranthaceae, and Balsaminaceae.

TESTS WITH SINGLE-LESION STRAINS OF THE VIRUS

Since Johnson (8, 9) showed that white clover mosaic is caused by a mixture of two viruses, a test was made to determine whether the yellow-patch disease was caused by a single virus or by a mixture of one or more viruses. A dilute suspension of the virus from tobacco was rubbed over Bountiful bean leaves. Single, well-isolated, local lesions that developed were punched out, macerated in a few drops of water, and rubbed over leaves of pepper. The symptoms produced in pepper by these single-lesion transfers were identical with those obtained previously in mass inoculations. Juice from infected peppers was sub-inoculated to tobacco. Typical symptoms of yellow patch developed. In a similar test, a single-lesion strain of the virus was carried from bean to tobacco, to periwinkle, then back to Ladino clover. Again, typical symptoms developed. These tests suggest that the disease in Ladino clover is caused by a single virus entity, not a complex.

ATTEMPTS TO TRANSMIT THE VIRUS WITH DODDER

Johnson (8, 9) found that one member of the white clover mosaic virus complex could be transmitted by dodder, *Cuscuta campestris* Yunck. To determine whether or not dodder could transmit yellow patch virus, strands parasitizing plants of Ladino clover infected with the virus were trained to healthy seedlings of Ladino clover. Despite connection of healthy and diseased plants for 6–8 weeks, no symptoms developed. When juice from seedlings of Ladino clover connected by dodder to infected plants was rubbed onto leaves of Bountiful bean, no local lesions or other symptoms of virus infection developed. Juice was then extracted from dodder parasitizing virus-infected plants and rubbed onto leaves of bean. No local lesions or other symptoms developed. These tests indicate that the yellow patch virus is not readily picked up or transmitted by dodder.

PROPERTIES OF THE VIRUS

To determine the thermal inactivation point for yellow patch virus, juices from infected plants of Ladino clover and of Turkish tobacco were heated in a water bath for 10 min. at various temperatures. Activity of the heated samples was determined by inoculation of from 4 to 24 primary

leaves of Bountiful bean seedlings. The data obtained indicate that the thermal inactivation point lies between 62° and 63° C.

In similar tests, solutions of the virus buffered at pH 7.0 were incubated at 18°–20° C. At intervals samples were removed and inoculated onto leaves of Bountiful bean seedlings. They produced from 25.7 lesions/ sq. cm. at 0 hours to 3.1 lesions/sq. cm. after 48 hours. At 28° C., the number of lesions produced on Bountiful bean was reduced 80 per cent in 60 minutes.

Virus activity was lost when the juice from infected Ladino clover plants was diluted beyond 1 : 100.

IDENTIFICATION OF YELLOW PATCH VIRUS

From symptomatology, host range, and virus properties, it is apparent that the yellow patch virus has much in common with strains of alfalfa mosaic virus. The virus produced local lesions on bean and mottling in tobacco resembling symptoms produced by alfalfa mosaic. According to Price (16), alfalfa mosaic virus infects a wide range of hosts. The yellow patch virus was capable of infecting hosts in 6 of the 9 families tested. Most of these hosts are known to be susceptible to alfalfa mosaic virus (6, 16, 19). Moreover, the thermostability of yellow patch virus is comparable to that of the type strain of alfalfa mosaic virus.

The yellow patch virus differs from other strains of alfalfa mosaic virus in several respects. Symptoms on tobacco distinguish it from the type strain, from the three strains studied by Zaumeyer (23), and from the strain isolated from pepper by Berkeley (3). Its tolerance to dilution and aging-in-vitro distinguish it from the strain reported by Pierce (13) and the strain that attacks celery reported by Snyder and Rich (17). Although symptoms in *Trifolium repens* produced by the yellow patch virus resemble those produced by a virus obtained from white clover by Zaumeyer and Wade (20, 21, 22), other properties of the two viruses are dissimilar. The strain of alfalfa mosaic virus from *T. repens* described by Valleau (18) and Johnson (10) possessed many of the characteristics of the yellow patch virus, but the thermal death point of Johnson's isolate was lower (55°–58° C.). Moreover, Johnson was unable to transmit his virus to white clover, zinnia, or pepper which were readily infected by the yellow patch virus. Ainsworth and Ogilvie (1) reported a virus-like disorder of white clover but were unable to transmit it to *T. repens* or other hosts tested.

Several additional viruses have been reported capable of infecting *Trifolium repens* L. but none of these are similar to the yellow patch virus. Although potato calico virus (4) infects many of the same hosts as the Ladino clover virus, the calico virus infects *Cucumis sativus* L. and yellow patch virus does not. Other properties of the two viruses also are dissimilar. Dickson (5) and Osborn (12) reported artificial infection of *T. repens* by a mosaic virus of red clover but failed to transmit the virus

to bean or tobacco. Johnson and Jones (7) infected *T. repens* with pea severe mosaic virus but were unable to transmit the virus to tobacco. Infectivity to tobacco distinguishes the yellow patch virus from several other virus diseases of *T. repens* (8, 9, 14).

On the basis of differences here reported it is concluded that the yellow patch virus is a strain of alfalfa mosaic different from any described previously.

Technical description

Marmor medicaginis H. var. **Ladino** n. var.

Common name: Ladino clover yellow patch virus. Differing from the type strain in symptomatology on tobacco and tolerance to dilution and aging-in-vitro.

Hosts: LEGUMINOSAE—*Trifolium repens* L. var. *Ladino*, Ladino clover. Also transmissible experimentally to many species of dicotyledonous plants including AMARANTHACEAE—*Celosia argentea* L. var. *cristata* Kuntze, cockscomb. APOCYNACEAE—*Vinca rosea* L., periwinkle BALSAMINACEAE—*Impatiens holstii* Engler and Warb., balsam. COMPOSITAE—*Zinnia elegans* Jacq., zinnia. LEGUMINOSAE—*Glycine max* (L.) Merr., soybean, *Phaseolus vulgaris* L., bean, *Pisum sativum* L., pea. SCROPHULARIACEAE—*Antirrhinum majus* L., snapdragon. SOLANACEAE—*Capsicum frutescens* L., pepper, *Nicotiana tabacum* L., tobacco.

Geographical distribution: United States.

Induced disease: In Ladino clover, tobacco, pepper, and periwinkle, systemic chlorotic yellow mottling, occurring mostly in patches. In varieties of garden bean, numerous reddish necrotic local lesions.

Transmission: By inoculation of expressed juice in dilutions up to 1:100. Not by dodder, *Cuscuta campestris* Yunck.

Thermal inactivation: At 62°–63° C. in 10 min., at 18°–20° C. in 48 hr.

DISCUSSION

Cross-protection tests with the type strain or with other strains of alfalfa mosaic virus would have aided in establishing the relationship of yellow patch virus to alfalfa mosaic virus, but none of these strains could be obtained for comparison. Despite this, the host range and virus properties demonstrate the close relationship of yellow patch virus to other described strains of alfalfa mosaic virus. The broad host range suggests that yellow patch virus may possibly be transmitted from other hosts to Ladino clover by an unknown insect vector. Seeds harvested from plants of Ladino clover infected with yellow patch virus have yielded disease-free seedlings. This indicates that the yellow patch virus is probably not seed-transmitted in Ladino clover.

Since winter survival is a major problem in growing Ladino clover and observations indicate that plants infected with yellow patch virus are weakened, there is a further possibility that diseased plants may succumb

to winter injury ordinarily not damaging to healthy plants. The increasing importance of Ladino clover as a forage legume and the corresponding increase in prevalence of yellow patch virus in this host suggest that measures for controlling or eliminating the disease should be developed.

SUMMARY

A disorder of Ladino clover referred to as yellow patch and characterized by chlorotic, yellow mottling of leaves is caused by a virus. Symptoms of the disorder were readily reproduced on a number of different hosts by rubbing juice from diseased plants of Ladino clover onto leaves of healthy plants. Tests with single-lesion strains of the virus demonstrated that the disease was caused by a single virus entity. The virus was not transmitted or taken up by dodder.

Virus activity was lost when juice from infected plants of Ladino clover was diluted beyond 1 : 100. A solution of the virus permitted to stand at 18°–20° C. was inactive after 48 hr. The thermal inactivation point for the virus was 62°–63° C.

From the host range and other properties, the virus was identified as a strain of alfalfa mosaic virus different from any described previously. The virus was given the trinomial *Marmor medicaginis* H. var. *Ladino* n. var.

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LABORATORY AND GREENHOUSE STUDIES OF ANTIMYCIN PREPARATIONS AS PROTECTANT FUNGICIDES[1]

CURT LEBEN AND G. W. KEITT[2]

With the remarkable successes in the use of antibiotics in the control of diseases of animals, there has been an increase in interest in the possibilities of use of such materials against plant diseases. A number of antibiotics have been employed in seed treatments (3, 4, 5, 9, 22, 23), in inducing artificial resistance to disease (3, 4), and in chemotherapy (4, 6, 7, 10, 20), with varying results. This laboratory has published preliminary reports of an antibiotic as a protectant fungicide under greenhouse conditions (15, 16). The purpose of the present paper is to report these and further investigations of this antibiotic.

MATERIALS AND METHODS

Antibiotic preparations. The antibiotic material treated in this report is produced by an unidentified species of *Streptomyces.* In a previous study (17) the production, properties, and biological activities of crude preparations were considered, and the name antimycin was proposed for the active material. Later (8) an active crystalline compound was isolated from these crude preparations. The name antimycin A was then proposed for this crystalline substance. There is evidence that the crude material contains one or more antibiotic factors in addition to antimycin A.

The preparations used in the present studies were derived from shake or tank cultures (8) of the antibiotic organism. Their antibiotic potency, in terms of units per ml., was determined by a plate assay using *Glomerella cingulata* (Stoneman) Spauld. and v. Schrenk as the assay organism (8). The active material was extracted with ethanol from the precipitate formed on the acidification of the culture filtrate. In the early experiments in the greenhouse this ethanol extract, which will be known hereinafter as "crude antimycin in ethanol," was used as a protectant spray. Later, aqueous suspensions were employed. These were made by removing the ethanol over water by reduced pressure distillation. The active material remained in the water as an insoluble, green-brown, fine precipitate that settled slowly. Unless otherwise noted these suspensions were used in all experiments reported in this paper; they are designated as "crude antimycin suspensions" or more simply as "suspensions." Their color, texture, rate of settling, and pH (usually 4.0 to 5.0) varied from fermentation to fermentation. Purity also varied: suspensions from seven to 58 units per mg.

(dry weight) were employed in the various studies. Crystalline antimycin A (ca. 1000 units per mg.) was used in the form of a microsuspension in water.

Foliage disease methods. In part of the work two foliage diseases, apple scab and tomato early blight, were used in the evaluation of the various antibiotic preparations under greenhouse conditions. Ordinarily, susceptible leaves were sprayed with test material, dried (usually 2–4 hr.), and inoculated with a spore suspension of the disease-inciting fungus, and the plants were placed under conditions favorable for the development of the disease. In some tests plants were washed with simulated rain after the antibiotic spray had dried. These plants were then allowed to dry before inoculation. In the incitation of apple scab, the methods of Keitt
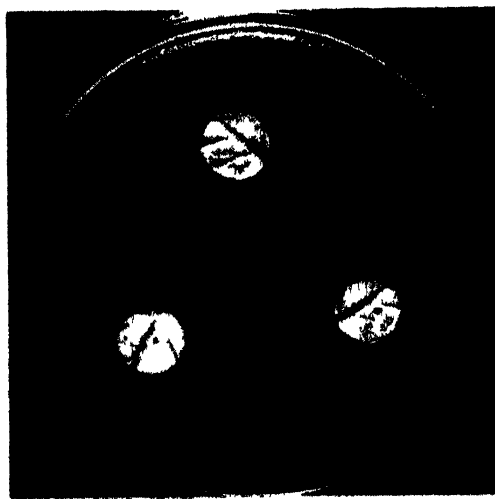
Fig. 1. Inhibition zones produced by leaf disks sprayed with crude antimycin suspensions.

and Langford (13) were used; in the incitation of early blight, those of McCallan and Wellman (19) were employed with certain exceptions.[3] Precision spray apparatus was not available; in all of the studies reported in this paper antibiotic preparation and spore suspensions were sprayed on the upper sides of the leaves, as uniformly as possible by hand, with a DeVilbiss No. 15 atomizer. Spraying was stopped when runoff was about to begin.

Leaf disk assay. A preliminary report on the use of this assay for determining the amount of antibiotic activity on leaves has appeared (14). The method depends on the diffusion of the antibiotic from treated leaf disks into an agar medium containing an assay fungus. The amount of antibiotic on the disks is estimated by comparing the resulting inhibition

[3] An isolate of *Alternaria solani* (E. and M.) Jones and Grout requiring no special treatment (18, 19) for the production of spores was used. In different series of experiments, plants were 5–8 in. high, and, depending on the series, lesions on 3 or 4 leaves were counted.

zones with those produced by known amounts of the antibiotic in blotting paper disks. Ordinarily plants were sprayed with the antibiotic preparations, dried (2–4 hr.), and leaf disk samples taken at random. In rain tests, additional samples were taken after the plants had been subjected to simulated rain and dried.

Problems of contamination were encountered in the development of the leaf disk assay. When leaf disks treated with crude antimycin suspensions replaced the blotting-paper disks in the regular assay (8), fast-growing bacteria from the disks usually obscured the inhibition zones. This difficulty was overcome by adjusting the assay medium from pH 6.8 to 4.0; however, under these conditions the edges of the zones were too indistinct to be measured accurately. Consequently, studies were made with various acidic media and a number of fungi that had been shown previously to be inhibited by the crude material. Ultimately a combination that gave suitable zones was selected (Fig. 1). Under the conditions of this work growth of bacteria was controlled and only occasionally did fast-growing fungi from the disks prevent measurement of zones. The assay procedure follows.

Fifteen ml amounts of acidified potato-dextrose agar[4] were allowed to harden in a series of Petri plates. These were then layered with 5 ml. of an agar spore suspension, which was made by mixing one volume of a distilled water spore suspension of *Colletotrichum circinans* (Berk) Vogl.[5] with 4 volumes of acidified potato-dextrose agar at 38–40° C. When the suspension had solidified, leaf disks 0.5-in. in diameter were cut from treated plants and placed, sprayed side downward, on the surface of the agar. Each disk was pressed gently to insure complete contact At the same time blotting-paper disks 0.5-in. in diameter containing different ethanol dilutions of a standard solution of crude antimycin in ethanol were also placed on the seeded medium. Disks from four treatments usually were put on one plate. Each treatment was replicated six times, each time on a different plate. Plates were incubated 40–48 hr. at 28° C. and the average diameter of the inhibition zones was determined. A curve was plotted with the log of the antibiotic potency of the standard as the abscissa and the diameter of the inhibition zone as the ordinate. An example of a standard curve is given in figure 2. The same curve was produced when the standard was tested at the beginning or at the end of the time (1–3 hr.) the leaf disks were being placed on the agar. The amount of antibiotic on the leaf disks was determined by interpolation (sometimes extrapolation) of the standard curve and expressed in "leaf units." One leaf unit is the amount of active material on a leaf disk that will produce an inhibition zone the same size as that produced by a blotting paper disk containing 1 unit per ml. of the standard. To standardize further the term "leaf unit,"

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[4] Per liter: extract from 200 gm. of sliced, peeled potatoes steamed 0.5 hr. in 500 ml. of water; glucose, 25 gm.; agar, 17 gm. The reaction was adjusted with 2N HCl immediately before the plates were poured so that the pH of the medium was 3.8–4.2.

[5] Our isolate 30. The water suspension contained about 100,000 spores per ml. Spores were removed from potato-dextrose-agar slants 7–14 days after seeding.

it will be necessary to specify the volume of the standard added to the blotting-paper disks; in the present studies one lot of blotting paper was used in making the disks, and they were all dipped and drained in a uniform manner.

Accuracy of the leaf disk assay is indicated by the average and standard deviation determined from several tests: 10.9 ± 1.6 (4 replicates), 40.5 ± 6.5 (6 replicates), 18.3 ± 1.4 (8 replicates), 26.1 ± 4.0, 11.5 ± 1.6, and 10.3 ± 1.6 (all with 10 replicates) leaf units. It is probable that accuracy can be increased by further study.

The leaf disk assay appears to be advantageous because of its relative
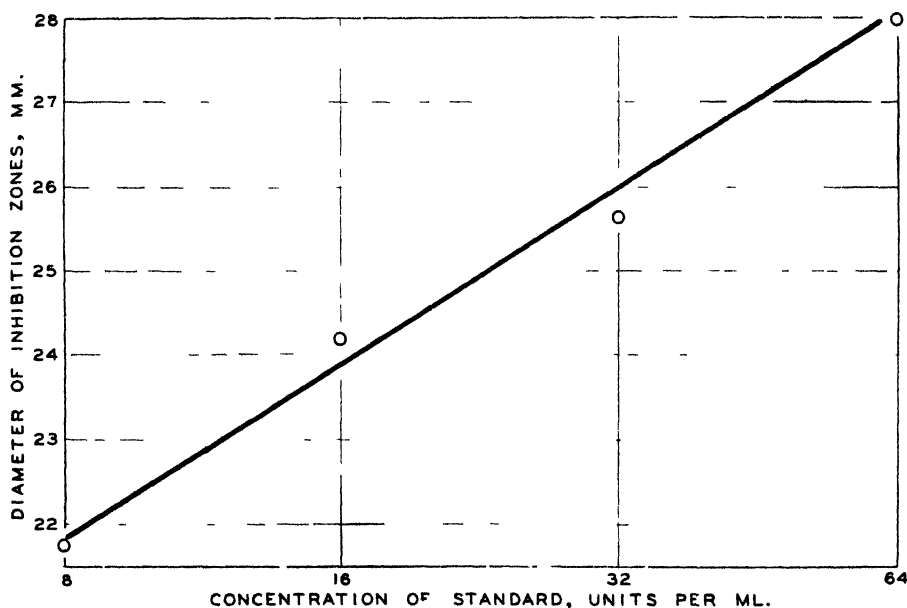
Fig. 2. The relation of the concentration of antibiotic in blotting-paper disks to inhibition zone size.

simplicity and because fungicide-plant interactions are taken into consideration. Preliminary studies indicate that with suitable modification the assay may be used for diffusible fungicides not of microbial origin. Leaves of tomato (*Lycopersicon esculentum* Mill.), apple (*Malus sylvestris* Mill.), cowpea (*Vigna sinensis* (L.) Endl.), elm (*Ulmus americana* L.), and *Lonicera tatarica* L. have been used in leaf disk assays. With these species no inhibition zones were produced unless leaves were first sprayed with a suitable fungicide.

Simulated rain tests. Simulated rain was produced by lake water flowing through a flat type hose sprinkler, which was directed slightly upward so that the water fell gently by gravity on one side of an electrically driven turntable. Test plants were placed toward the edge of the turntable so that on each revolution each plant passed through a zone of simu-

lated rain. The turntable, which was 4 ft. in diameter, made 4 revolutions per min.; the water fall per minute on any given plant was approximately 1 in.

Slide-germination methods. A precision horizontal sprayer modeled after that described by Horsfall (11) was used to note the inhibiting effects of various antimycin preparations on spores of *Glomerella cingulata.* Routine methods (1) were used. Tests were made at 28° C., and filtered orange juice was employed as a stimulant.

EXPERIMENTAL RESULTS

The protective effect. Crude antimycin in ethanol was first shown to be effective as a protectant in greenhouse tests with *Venturia inaequalis* (Cke.) Wint. The results of typical experiments are given in table 1.

TABLE 1.—*The effect of crude antimycin in ethanol on infection of apple leaves by Venturia inaequalis*

Experiment number	Test shoots[a]		Control shoots[c]	
	No.	Symptoms[b]	No.	Symptoms
1	8	4 flecks	6	150 lesions
2	6	none	6	150 do
3	10	2 lesions, 6 flecks	8	1000 do

[a] Shoots sprayed with antibiotic solutions. Shoots in experiment 1 sprayed with one preparation (1 unit per ml.) and shoots in experiments 2 and 3 sprayed with another preparation (2 units per ml.).
[b] Lesion is the typical symptom; fleck is a reduced chlorotic area, usually with no sporulation.
[c] Sprayed with ethanol.

Under some conditions a marked distortion or killing of the upper leaves was noted, caused by the ethanol in which the antibiotic was carried. Water suspensions or 25 per cent ethanol suspensions did not induce this injury when used in the control of apple scab or early blight (Table 2).

TABLE 2.—*The effect of a crude antimycin suspension on the control of tomato early blight*

Potency of spray in units per ml.	Lesions per plant[a]	
	Test 1	Test 2
No.	No.	No.
12	0	0
3	0	0
0	69	61

[a] Single plant tests except for controls. Control for test 1, average of 3 plants; for test 2, average of 2 plants.

Consequently, aqueous suspensions were used in subsequent experiments.

In later studies a dosage-response curve was plotted for early blight (Fig. 3).

Deposition. In a series of studies with the leaf disk assay, several factors were found to influence the amount of antibiotic deposited. The deposit varied with different species of plant. For example, the leaf unit values produced by one antibiotic suspension with tomato, cowpea, *Lonicera tatarica,* and elm were, respectively, 16.5, 9.3, 9.8, and 8.0. Differences in the deposit were noted also with various suspensions although they were
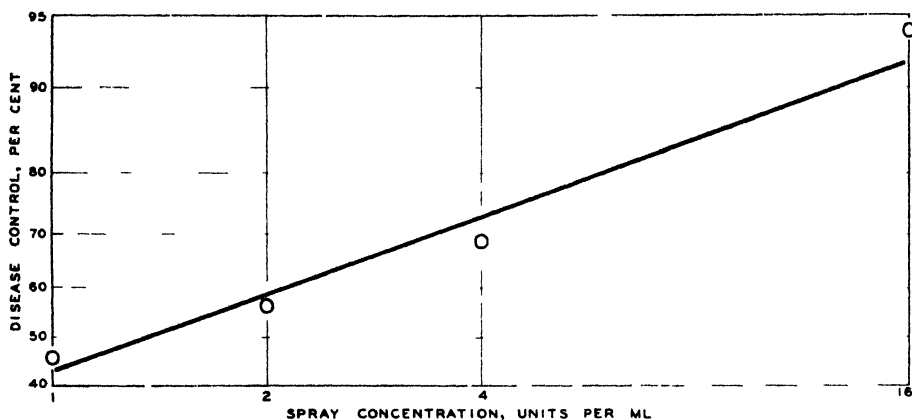
FIG. 3. Dosage response curve for a crude antimycin suspension against tomato early blight. Single plant tests were repeated in experiments completed at different times. Each point is the average of 3 tests, except at 2 units per ml., where there were 2 tests.

of approximately the same antibiotic potency Certain insecticides, surface-active compounds, or stickers also influenced the amount of antibiotic retained on the leaf. Probably other factors such as humidity, temperature, and age and condition of the plant are also important.

TABLE 3.—*Effect of different time intervals between spraying and inoculation on the control of apple scab by a crude antimycin suspension*

Hours spray applied before inoc.	Spray material[a]	Lesions per shoot[b]	Disease control
No.		No.	Per cent
96	Test	13.0	78.4
Do	Control	60.1	
48	Test	1.8	95.7
Do	Control	42.5	
4	Test	0.0	100.0
Do	Control	81.0	

[a] Test spray: 3 units per ml. carried in 25 per cent ethanol; control spray: 25 per cent ethanol.
[b] Average of 10 test shoots or 4 control shoots.

Stability on plant leaves. Greenhouse studies with *Venturia inaequalis* indicated that one antibiotic suspension lost some activity after 96 hr. on apple leaves (Table 3). With another suspension, a loss in activity on tomato and apple leaves was noted (Table 4): approximately one half of the original activity remained after 4 days. On tomato leaves part of

TABLE 4.—*The stability of crude antimycin on plant leaves*

Test[a]	Activity in leaf units on leaves after:		
	4-6 hours	2 days	4 days
	No.	No.	No.
1	13.0	9.5	6.2
2	15.0	12.4	7.2
3	12.4	12.0	7.5

[a] Tests 1 and 2 made with tomato leaves, test 3 made with apple leaves. The same crude antimycin suspension was used in all tests.

this reduction was probably due to an increase in leaf size; however, the increase in size of the apple leaves was negligible.

Effect of simulated rain. The effect of washing various preparations

TABLE 5.—*Effect of simulated rain on the control of apple scab by a crude antimycin suspension*

Potency of spray in units per ml.	Disease control[a]	
	Unwashed shoots	Washed shoots[b]
No.	Per cent	Per cent
4	81.7	59.2
2	77.5	66.2
1	36.6	25.4
0.5	2.9	0.0
0.25	0.0	0.0
1.125	0.0	0.0

[a] Each entry average of 8-10 shoots.
[b] Simulated rain, 2 inches.
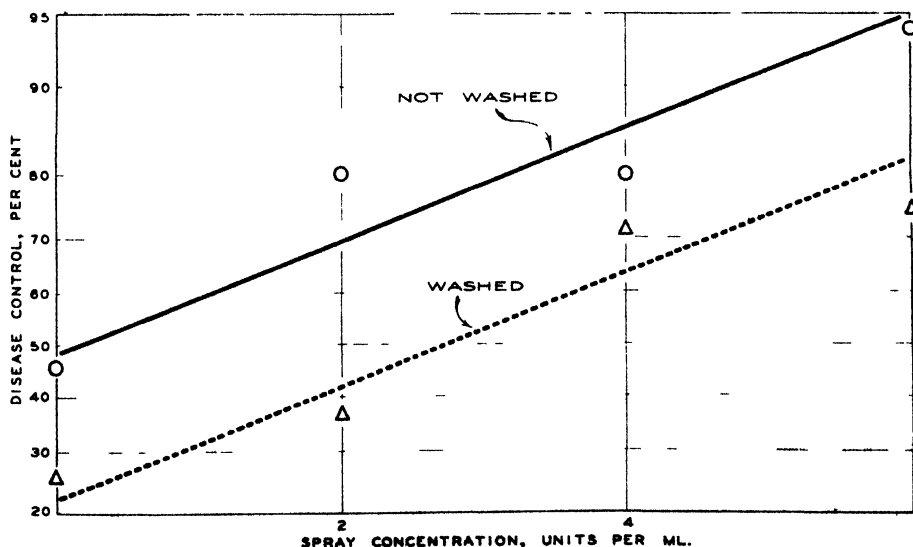
FIG. 4. Effect of simulated rain on the control of tomato early blight by one crude antimycin suspension. Single plant tests repeated in experiments completed at different times. Each point is the average of 3 tests.

with simulated rain was studied with the aid of the foliage disease methods
and the leaf disk assay. In two foliage disease tests, different suspensions

TABLE 6.—*Effect of simulated rain on the washing of different antimycin preparations
from tomato leaves*

Preparation No.	Preparation type	Spray potency in units per ml.	Amount removed by 2 in. of rain
		No.	Per cent
165	Crude suspension	14	72.3 ± 6.6[a]
169	do	15	95
174	do	13	99
175	do	8	100
183	do	30	82, 87[b]
Do	do	60	94, 95[c]
B-8-18	d	12	93
B-8-43X	Crystalline antimycin A suspension	15	98, 100[b]

[a] Data from 6 experiments, 5 of which were completed at different times.
[b] Results from 2 experiments completed at different times.
[c] Results from 2 experiments completed at same time.
[d] Crude antimycin suspension partly purified (material derived from ether extract
(8) suspended in water).

were indicated to be relatively resistant to washing (Table 5, Fig. 4). In
another series of early blight tests with still another suspension, there appeared to be little or no washoff. On the basis of these tests it was re-
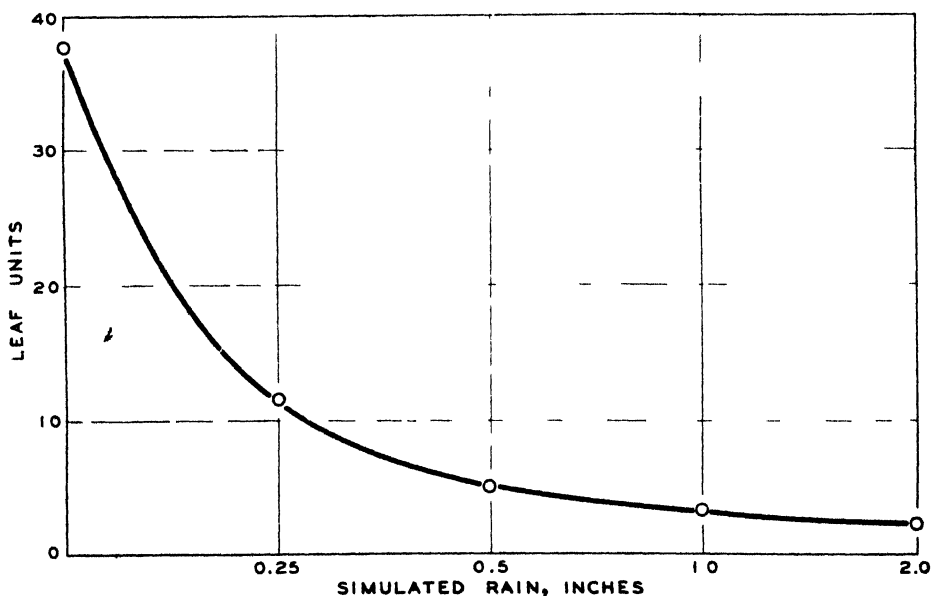
FIG. 5. Antibiotic activity remaining after tomato leaves were washed with various amounts of simulated rain.

ported that the crude material was resistant to washing (16). These results also suggested that some suspensions were more resistant to washing
than others. Data from leaf disk assays substantiated this (Table 6).

With one suspension (No. 183, 60 units per ml.) most of the washoff occurred with the first ¼ in. of rain (Fig. 5). The washoff of the most resistant preparation was comparatively high (72.3 per cent). However, when the dosage-response curve is considered (Figs. 3, 4), such a reduction does not necessarily result in a corresponding reduction in percentage of disease control. It is concluded from these experiments that some antimycin preparations are comparatively resistant to washing and that others are not.

Several materials that have been used as stickers were tested in attempts to reduce washoff. Data obtained with leaf disk assays are given in table 7. No adjuvant appeared to have any marked effect in prevent-

TABLE 7 —*Effect of certain adjuvants on the removal of crude antimycin suspensions from tomato leaves by simulated rain*

| Adjuvant | Amount of activity removed by 2 in. of rain | |
	Prep. 165[a]	Prep. 183[b]
	Per cent	Per cent
PEPS[c]	81	76.9
Vulcanized rapeseed oil[d]	84	83.6
Soybean oil emulsion[e]	72	
None	71	82.0

[a] Average of 3 tests completed at different times.
[b] Results from 1 test.
[c] Polyethylene pentasulphide, 1 in 400 (21). Courtesy of B. F. Goodrich Chemical Co.
[d] 1 lb. per 100 gal (12). Courtesy of the Stamford Rubber Supply Co.
[e] Soybean oil (0 25 per cent) emulsified with the aid of gum ghatti (0.005 per cent).

ing washoff. Under different drying conditions or with repeated applications, perhaps washoff would be lessened.

Effects of certain insecticides. The mixing of certain insecticides with crude preparations did not indicate loss of antibiotic activity. The insecticides tried were 50 per cent wettable DDT (DuPont), 2 lb. per 100 gal.; benzene hexachloride (Eastman, Pract.), 1 lb. per 100 gal.; hexaethyltetraphosphate (Planetary Chemical Co., Blot), 1 pint per 100 gal.; nicotine sulphate (Tobacco By-Products and Chem. Corp., Black Leaf 40), 2.5 pints per 100 gal., and lead arsenate, 2 lb per 100 gal.

In one series of tests, the effects of these insecticides on the antibiotic material on the plant were investigated. A crude antimycin suspension was sprayed on a series of tomato plants (6–8 in. high) and the plants were dried; then sprays of the various insecticides were applied to separate plants. The two applications were made to avoid variations in deposit encountered when the antibiotic and insecticides were mixed before spraying. After 24 hr., leaf disk assays were made. Results indicated that as much antibiotic remained on leaves sprayed with various insecticides as on those that received no insecticide.

It is therefore concluded that under the conditions of these tests the insecticides had little if any effect on antibiotic activity. The effects of the

antibiotic preparations on the insecticidal properties of the various compounds are not known.

Slide-germination tests. In tests with *Glomerella cingulata,* the LD 50 for one crude suspension (8.1 units per mg.) was found to be 1.07 μg. per cm.^{2} and that for standard Bordeaux (2) was 0.68 μg. per cm.^{2} (Fig. 6). On the basis of purity, expressed as units per mg., the predicted LD 50 for crystalline antimycin A was estimated to be *ca.* 0.008 μg. per cm.^{2} In actual tests, however, the LD 50 was more than 0.4 μg. per cm.^{2}, the highest concentration that was available. This is an additional evidence
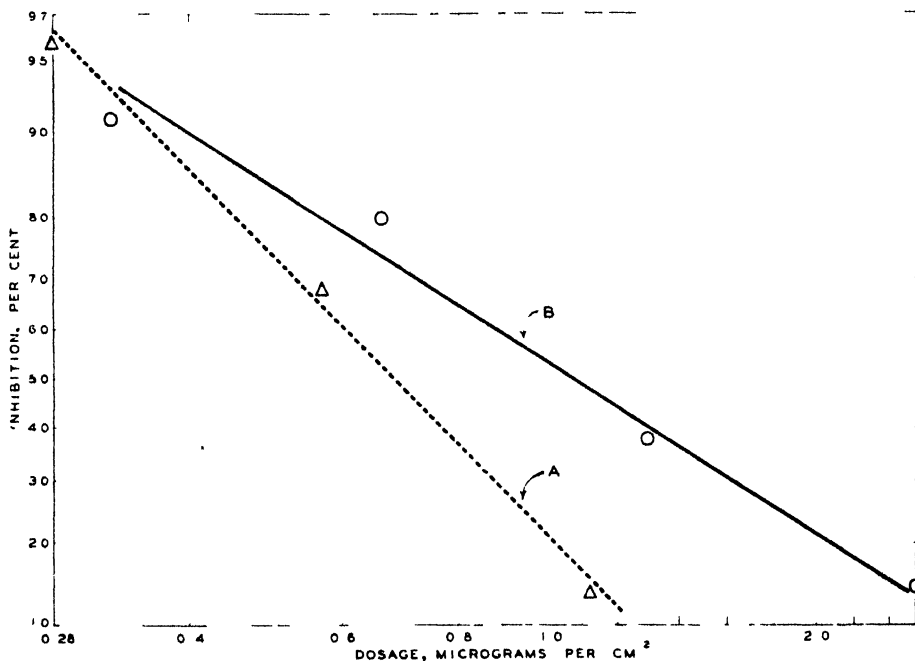
FIG. 6. Dosage response curves for standard Bordeaux mixture (A) and one crude antimycin suspension (B) as determined by slide-germination tests using *Glomerella cingulata.*

(8) that inhibiting substances other than antimycin A may be found in the crude preparations.

Phytotoxicity. No phytotoxic effects have been noted with aqueous sprays of 100 units per ml. of the crude or crystalline suspensions on young tomato, bean (*Phaseolus vulgaris* L.), cucumber (*Cucumis sativus* L.), or cowpea plants under greenhouse conditions. Less potent crude suspensions have been sprayed on apple and pea (*Pisum sativum* L.) plants with no injury. No visible residues have ever been noticed.

The toxicity to man or experimental animals is not known, although studies on this subject are under way. No toxic effects have been observed by any of the people concerned with the various phases of the work.

DISCUSSION

In the present study it was demonstrated that antibiotic preparations derived from cultures of an unidentified *Streptomyces* species were effective as protectant fungicides in the greenhouse. Similar preparations from different fermentations of this organism, however, differed markedly in a number of properties and in disease controlling potentialities.

The effective preparations varied in texture, purity, amount retained on the leaf, resistance to washing with simulated rain, etc. The amount of activity (units per ml.) required for disease control also appeared to differ with various preparations (Tables 1, 2, 3, 5, and Figs. 3, 4), although these variations possibly may have been associated with differences in deposit or with experimental conditions that could not be controlled with the available facilities. Whether these variations in the crude preparations are caused by impurities, by antimycin A, or possibly by additional antibiotics is not known. However, the capacity to withstand washing did not appear to be associated with antimycin A, since this material was washed readily from the leaves

It is concluded from these investigations that certain preparations of antimycin have possibilities as protectant fungicides. Small amounts were effective and were compatible with insecticides. The material was comparatively resistant to washing, and there were no visible residues. Phytotoxicity did not appear to be important On the other hand, loss of activity on the leaf has been noted, and little is known about the toxicity of the material to man. The problem of differences in various preparations appears to be one of antibiotic production and control—a problem that seems solvable and one that is not peculiar to the fermentation industries.

The investigation thus far has been more concerned with the potentialities of antibiotic materials for combating plant diseases than with the possible practical adaptations of antimycin as a protectant fungicide. The high antibiotic effect of the various preparations on many fungi and their efficiency in the control of apple scab and tomato early blight in the greenhouse give encouragement to further studies of these preparations and of other antibiotics in relation to plant disease control.

SUMMARY

Various crude antibiotic preparations derived from cultures of an unidentified species of *Streptomyces* were effective as protectant fungicides in controlling apple scab and tomato early blight in the greenhouse. Preparations made from different fermentations varied in a number of properties, including the capacity to withstand washing from leaves. Certain crude preparations were comparatively resistant to washing, whereas crystalline antimycin A, derived from the crude material, was washed readily from the leaves. With the passage of time a loss in antibiotic activity on plant leaves was observed. No phytotoxicity was observed, and

crude preparations appeared to be compatible with a number of insecticides.

The leaf disk assay for determining the amount of antibiotic on leaves is described.

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STUDIES ON THE MECHANISM OF RESISTANCE OF CRUCIFEROUS PLANTS TO PERONOSPORA PARASITICA[1]

T . M . W A N G [2]

An understanding of the mechanism of resistance is of prime importance in the breeding of plants resistant to disease. A comparative study was made of the host-parasite relationships and the environmental effects on the development of downy mildew in susceptible and resistant varieties of cruciferous plants, so as to throw light on the nature of their resistance to the fungus *Peronospora parasitica* (Pers.) de Bary.

MATERIALS AND METHODS

Most of the experimental work was done in Chengtu, China, from 1943 to 1944, and part of it was repeated in Nanking in 1947. Cruciferous varieties with different degrees of susceptibility or resistance were chosen. namely, Chinese rape (*Brassica Chinensis* L.), cabbage (*B. oleracea* L.), and radish (*Raphanus sativus* L.). The inoculum used consisted of the sporangia of *Peronospora parasitica brassicae* race 2 and *Peronospora parasitica raphani* (5). Leaves from which inoculum was to be taken were thoroughly washed in water so as to remove sporangia that had formed and also any surface contaminants that might be present. They were then placed in a moist chamber to secure a new crop of sporangia for inoculum. The spore suspension for inoculation was made by brushing sporangia into sterile tap water with the aid of a clean brush. Spore suspensions were atomized on the surfaces of detached leaves or of seedlings, and the inoculated leaves were kept in a moisture-saturated atmosphere for 1 day or longer. Light was admitted into the moist chamber through the glass cover. The inoculated plants, after remaining in the moist chamber for 1 or more days, were taken out and kept in the greenhouse while the disease developed.

PENETRATION BY THE PATHOGEN

Tsu (4) reported that the germ-tubes of sporangia of *Peronospora parasitica* penetrate the epidermis of the host directly and invade the interior through intercellular spaces; but he does not mention the method of infection on resistant varieties. An effort was made in the present experiment to find out whether the germ-tube penetrates resistant and immune plants.

Chinese rape, cabbage, and radish were chosen for such a test. Chinese rape is susceptible to race 2 of *Peronospora parasitica brassicae*, cabbage is resistant, and radish is immune. Chinese rape is immune from *P. parasitica raphani*, cabbage is resistant, and radish is susceptible to it. Cross-inoculations were made on detached leaves with *Peronospora parasitica brassicae* race 2 and *P. parasitica raphani*. One day after inoculation, pieces of epidermis were stripped from the inoculated leaves with a forceps, stained with cotton blue, and observed under a microscope.
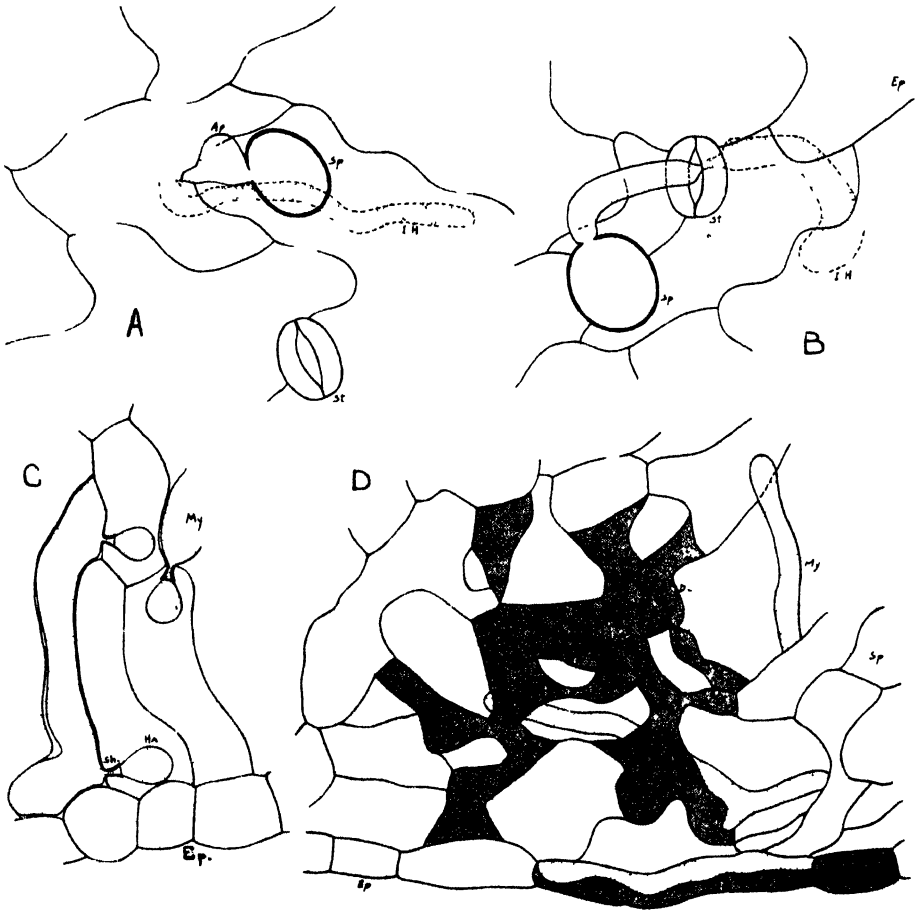
FIG. 1. *Peronospora parasitica brassicae* race 2. Above, entry of germ tube of the sporangium, through (A) an epidermal cell and (B) a stoma. Below, mycelium in tissue of (C) the susceptible Chinese rape host, and (D) the immune radish host. Legend: Sp, sporangium; Ap, appressorium; IH, infection hypha; My, mycelium; Ha, haustorium; Sh, sheath; Ep, epidermis; St, stoma; Sp, spongy mesophyll tissue; DC, dead host cells.

Both direct penetration and stomatal entry of the germ-tube were clearly observed on susceptible hosts, on the resistant hosts, and even on immune ones. In the course of direct penetration, the tip of the germ-tube swelled to form an appressorium. The infection hypha growing from the appressorium was greatly constricted as it penetrated the epidermal

cell with which it came in contact (Fig. 1, A). In the case of stomatal entry, the germ-tube of the sporangium also was constricted during the passage through a stoma (Fig. 1, B). Direct penetration was more frequently observed than was entry through a stoma. On the resistant and immune varieties, both types of penetration occurred. Later development of the infection hypha was greatly restricted on such hosts.

DEVELOPMENT OF THE PATHOGEN IN HOST TISSUE

There is a great diversity in the development of the pathogen after its entry into the plants, depending upon the degrees of their susceptibility. In the susceptible host, the invaded parts became discolored after 5 to 6 days, and the lesions gradually increased in size. On the resistant variety, only small necrotic spots, rather restricted in size, appeared on infected parts. Growth of the mycelium was greatly suppressed, an observation verified by study of stained sections of host tissue.

Healthy, detached leaves of Chinese rape and radish nourished in 5 per cent glucose solution were inoculated with sporangia of *Peronospora parasitica brassicae* race 2 and *P. parasitica raphani*, respectively. One set of Chinese rape and radish leaves was provided as a control in which no inoculation was made. Inoculated portions of leaves were fixed in Karpechenko's solution 2, 4, and 6 days after inoculation; the paraffin method was used for making sections, which were then stained with safranin and light green or haemotoxylin. Direct observation of leaves cleared with chloral hydrate and stained with cotton blue was sometimes used.

A series of observations revealed significant differences in the development of the infection hypha after the invasion of susceptible and immune hosts. After the infection hypha of *Peronospora parasitica brassicae* race 2 penetrated the susceptible rape host, the mycelium spread in all directions without limitation through the intercellular spaces of the leaf mesophyll, and at the same time sent globular or pyriform haustoria into the host cells. The base of each haustorium was usually surrounded by a sheath or membrane apparently of host origin (Fig. 1, C). On the other hand, in the immune radish variety the host cells surrounding the infection locus of *P. parasitica brassicae* race 2 became necrotic within 4 days after inoculation. A short hypha spread from the point of ingress without forming haustoria; in some cases hyphae apparently pushed against the host cells but failed to penetrate. Surrounding the invading mycelium were the dark brown, deformed, and dead host cells (Fig. 1, D). Similar phenomena were observed in the host tissues of radish and rape when inoculated with *P. parasitica raphani*. Apparently the resistance of cruciferous plants to *P. parasitica* is due to the death of the host cells, which prevents the establishment of the pathogen in the host tissue.

SPORULATION

An attempt was made to determine whether there were any differences in fungus sporulation on the lesions of susceptible and resistant hosts.

Cross-inoculations with sporangia of *Peronospora parasitica brassicae* race 2 and *P. parasitica raphani* were made separately on Chinese rape, cabbage, and radish grown in pots and also on their detached leaves placed in Petri dishes with moistened paper at the bottom.

Race 2 of *Peronospora parasitica brassicae* sporulated abundantly on infected seedlings of Chinese rape exposed for 1 day to a saturated atmosphere. Necrotic spots without fungus sporulation appeared on cabbage, and there was no visible sign of infection on radish seedlings. The *P. parasitica raphani* sporulated abundantly on radish seedlings but produced only necrotic spots without spores on cabbage seedlings. There was no sign of infection on rape seedlings.

Results were entirely different on detached leaves. Neither fungus sporulated on detached leaves of the susceptible hosts. On the hosts that were usually immune from attack, each fungus developed sufficiently to cause necrotic spots.

A similar experiment was made with detached leaves nourished in a 5 per cent glucose solution, Knop's solution, or tap water and placed near a window to receive light. Again necrotic spots appeared on the hosts that were usually immune. The fungi sporulated, however, on the susceptible hosts.

EFFECTS OF ENVIRONMENTAL FACTORS ON THE DEVELOPMENT OF THE DISEASE

An attempt was made to determine the environmental factors that might cause a change in the expression of symptoms of the host. In the inoculation experiments, particularly with detached leaves of immune varieties, black necrotic spots appeared under conditions of weak light and prolonged moisture. Consequently, two factors, light and moisture, were tested separately for their effects on the host reactions.

Effect of Light

The downy mildew caused by an obligate parasite was particularly sensitive to light. Light probably affects the host first and then in turn affects the development of the pathogen. Schaffnit (3), Forward (1), and Hart and Zaleski (2) have proved that the expression of rust in a susceptible host is more or less altered under weak light or in darkness.

Seedlings of Chinese rape and radish were inoculated with *Peronospora parasitica brassicae* race 2 and *P. parasitica raphani*. Twelve pots of each host were used for each fungus and were held for 1 day in the inoculation chamber. Then two pots of Chinese rape and two of radish were placed in normal sunlight in the greenhouse, while all other pots were removed to a room with weak light. Each day, sets of two pots of Chinese rape and radish seedlings were transferred from the weak light to normal sunlight.

Conspicuous necrotic spots appeared on the immune hosts in the weak light (Table 1), and such spots became more obvious and also more nu-

TABLE 1.—*Effect of weak light on the infection of Chinese rape and radish seedlings by Peronospora*

Inoculum and number of days in weak light	Infection on Chinese rape		Infection on radish	
	Percentage	Type[a]	Percentage	Type[a]
P. parasitica brassicae race 2				
0	100	S	0	I
1	100	S	21	NI
2	99	S	19	NI
3	100	S	46	R
4	100	S	95	R
5	100	S	100	R
P. parasitica raphani				
0	0	I	89	S
1	0	I	74	S
2	26	R	85	S
3	83	R	94	S
4	86	R	93	S

[a] I indicates immunity and no signs of infection; NI, near immunity and a few small necrotic spots; R, resistance and conspicuous necrotic spots; S, susceptibility and sporulation of the fungus.

merous as the time in weak light increased. On susceptible hosts each fungus developed and sporulated well regardless of the light conditions. The incubation period on all hosts generally was increased by 1 to 2 days when the illumination was weak.

Effect of Moisture

Sets of Chinese rape and radish seedlings were kept at a high atmospheric moisture so that their cells would be very turgid. They were inoculated with the two pathogens and kept in the moist chambers for varying times. The effect of light could not be completely eliminated because light within the moist chamber was always weaker than the normal sunlight of the greenhouse.

A high percentage of infection was always secured on the susceptible hosts and the fungus developed and sporulated normally regardless of whether the period of high moisture following inoculation was 1 day or 5 days. The incubation period usually was shortened by 1 or 2 days when moisture was high. A single day at high atmospheric moisture did not alter the immune reaction of radish seedlings for *Peronospora parasitica brassicae* race 2; but 2, 3, 4, or 5 days of high moisture following inoculation permitted the development of 3 to 11 per cent infection and the appearance of a few small necrotic lesions which changed the reaction to one of near immunity. With 5 days of high atmospheric moisture following inoculation, 2 per cent infection of *P. parasitica raphani* developed on rape seedlings and the reaction was a near immune reaction. On the whole, moisture probably was a less important factor than light in changing the host reaction to these pathogens.

DISCUSSION

The mechanism of resistance was studied by comparative observations of the pathogen's entry, its mycelial and haustorial development, and its sporulation in susceptible and resistant or immune hosts. None of the hosts hinder entry of the pathogen because the germ tubes of the fungus either penetrate the epidermis directly or enter the stomata without difficulty. After entry, however, there is great diversity in development of the fungus. In a susceptible variety the mycelium spreads freely in all directions through the intercellular spaces of the leaf mesophyll, and numerous haustoria enter the host cells to nourish the pathogen. In a resistant or an immune variety, the host cells surrounding the invading hyphae apparently become intoxicated and die. Consequently the fungus encounters difficulties in forming haustoria, and it may die from starvation or possibly because of toxins from the dead host cells. The only real difference between resistance and immunity lies in the quantity of host cells killed. In an immune host, so few cells may be killed that no external signs of infection are evident; in a near-immune host, the dead host cells are sufficiently numerous so that a few small necrotic lesions appear; and in a resistant host even more of the host cells are killed and numerous necrotic spots occur on the infected plant. The pathogen sporulates rather easily in susceptible hosts, but not in resistant or immune hosts.

The abundance of sporangia in lesions on a susceptible host implies that the fungus obtains a large amount of nutrient from the host. Yarwood (6) reported that downy mildew infection and sporulation of the fungus might cause a 55 per cent decrease in fresh weight of onion leaves, a similar decrease of 48 per cent in spinach leaves, and a decrease of 17 per cent in hop leaves. Sporulation during a single night might result in a decrease of 5 per cent in the dry weight of infected leaves (6). No such depletions of host materials are to be expected in resistant or immune varieties on which the fungus does not sporulate.

Environmental factors also play a role in the development of downy mildew. Light seems to be a more important factor than atmospheric moisture because a weak light modifies the immune reactions of a host. Weak light allows a somewhat greater development of fungus mycelium with the result that numerous and conspicuous necrotic lesions may appear on a host that generally has very few or no external symptoms of disease in normal sunlight. Nevertheless, the variability in disease reaction of the resistant hosts never was sufficient to allow the pathogen to sporulate, and the resistance is considered a true inherent resistance which plant breeders may depend upon for producing disease-resistant varieties.

SUMMARY

Host-parasite relationships were observed by means of inoculation studies and histological material for *Peronospora parasitica brassicae* race 2 and *P. parasitica raphani* on three hosts, Chinese rape, cabbage, and

radish. Chinese rape is susceptible to the first fungus and immune from the second, while a reciprocal relationship exists for radish. The cabbage host is resistant to both fungi.

The pathogens enter all plants whether susceptible, resistant, or immune, by penetrating directly through the epidermal cells or by entering the stomata.

After penetration, the fungus mycelium easily grows through the intercellular spaces of the leaf mesophyll and sends haustoria into the host cells of a susceptible host. In the resistant and immune hosts, development of mycelium and formation of haustoria are curtailed and the surrounding host cells die.

The fungi sporulate abundantly on susceptible hosts, but necrotic spots characterize the infection on resistant hosts. No symptoms or only a few small necrotic spots appear on the immune hosts.

Weak light may alter the immune reaction of a host so that it changes to a near immune or a resistant reaction. The differences are due primarily to the number of host cells killed by the invading fungus.

High atmospheric moisture may alter slightly the immune reaction of a host, but moisture is a less important environmental factor than light.

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GENETICS OF CERATOSTOMELLA. I. STRAINS IN CERATOS-TOMELLA FIMBRIATA (ELL. & HALS.) ELLIOTT FROM SWEET POTATOES[1]

E. O. OLSON

INTRODUCTION

The fungus *Ceratostomella fimbriata* (Ell. & Hals.) Elliott is the cause of the important field and storage disease of sweet potatoes known as black rot. The fungus is also of interest from a genetic standpoint since it belongs in a genus in which species have been reported as homothallic, heterothallic, or containing both homothallic and heterothallic strains.

While *Ceratostomella fimbriata* has been considered a homothallic species, self-sterile or nonperithecial cultures have been observed. In Louisiana such cultures were frequently obtained by L. H. Person while making routine isolations from black-rot-affected sweet potatoes. This indicated that there were different strains of this fungus.

The present studies were made to determine the occurrence of strains in *Ceratostomella fimbriata* and to find out how these strains differ. A brief report of part of this work has been made (21).

LITERATURE REVIEW

Previous studies of the genetics of various species of *Ceratostomella* have dealt primarily with the inheritance of the factors governing the perithecial stage. Certain species have been reported as heterothallic, *C. coerulea* Munch (20), *C. multiannulata* Hedge. & Davidson (9), *C. paradoxa* (de Seynes) Dade (8), *C. pluriannulata* Hedge. (14), *C. radicicola* Bliss (5), and *C. ulmi* Buisman (6, 23), and others, including *C. adiposum* Sartoris (22) and *C. quercus* Georg. (20), as homothallic. The species *C. fagi* Loos may consist of both homothallic and heterothallic strains. Loos (18) observed that single ascospore cultures produced perithecia but Buisman (7) found that single conidium cultures fell into two self-sterile groups and it was necessary to pair members of these two groups to obtain perithecia. Leach (16) observed that single ascospore cultures of *C. ips* Rumb. produced both perithecial and nonperithecial strains. When conidia were isolated from the perithecial strain, cultures of both strains were obtained. Conidia from the nonperithecial strain produced only the nonperithecial strain and cultures of the nonperithecial strain did not form perithecia when paired.

Ophiostoma multiannulatum (Hedge. & Davidson) Melin & Naunf., synonymous with *Ceratostomella multiannulata*, has been the subject of physiological and genetic studies by Fries (12) and Fries and Trolle (13). They reported that three nonallelic genes control the reduction of sulfates.

C. ulmi seems to consist of a number of strains which differ in cultural characters and pathogenicity (24, 25).

Various studies on the cytology of *Ceratostomella fimbriata* have been reported. Elliott (11) inferred that this species had heterothallic tendencies, while Mittman (20), Andrus and Harter (2, 3), and Gwynne-Vaughan and Broadhead (15) considered the fungus homothallic. Andrus and Harter (3), however, did isolate nonperithecial cultures. Barnett and

FIG. 1. A. Perithecium of self-fertile 1 strain of *Ceratostomella fimbriata* with typical long neck. B. Perithecium of self-fertile 4 strain with short neck. C. Sclerotium-like bodies in self-sterile 1 strain.

Lilly (4) reported that thiamin was necessary for perithecium production by *C. fimbriata*.

DESCRIPTION OF STRAINS

In the course of the present studies, strains were isolated which were self-fertile like the original parent, but which differed in other characters. Also, some strains were isolated which produced no perithecia when grown alone, and these likewise differed in certain other characters. For convenience, the ten strains reported in this paper are divided into two groups, self-fertile and self-sterile. The descriptions of each of the ten strains, based on growth on potato-dextrose agar, are given below.

Self-Fertile Strains

Self-fertile 1 was the wild type strain, which usually was isolated from black-rot lesions on sweet potatoes. It produced perithecia with long necks (Fig. 1, A) and abundant endoconidia.

Self-fertile 2 was similar to self-fertile 1 except that it grew more slowly, and the mycelium in culture had a bluish cast. Perithecia were beaked but tended to clump together. It was obtained as a variant from a cross between the strains self-fertile 4 × self-sterile 1.

Self-fertile 3 was similar to self-fertile 1 but grew faster and produced more aerial mycelium. Endoconidium production was perhaps slightly less than in the wild type. It occurred as a variant from the cross self-fertile 4 × self-sterile 2.

Self-fertile 4 appeared as a variant in isolations from the perithecia formed by pairing self-fertile 1 and self-sterile 1. It grew faster than self-fertile 1 in the early stages of growth and produced more aerial mycelium. Endoconidia were abundant. The salient characteristic of this strain was the production of short-necked perithecia (Fig. 1, B), which were fimbriate, like those of the other self-fertile strains.

Self-Sterile Strains

Self-sterile 1 produced sclerotium-like bodies, probably protoperithecia (Fig. 1, C), in culture, and an abundance of endoconidia. It was culturally similar to self-fertile 1. It was first isolated from black-rot lesions on sweet potatoes and occurred constantly among the ascospore progenies of self-fertile 1.

Self-sterile 2, similar to self-sterile 1 in that sclerotium-like bodies and abundant endoconidia were produced, was characterized by a slower growth rate and the bluish cast of the cultures. It was obtained constantly from the ascospore cultures of self-fertile 2.

Self-sterile 3 produced sclerotium-like bodies, grew faster than the two previous self-sterile strains, had more aerial mycelium, and produced fewer endoconidia. It was obtained from the ascospore progeny of self-fertile 3.

Self-sterile 4 produced no sclerotium-like bodies and formed few endoconidia. It was consistently found among the ascospore cultures of self-fertile 4.

Self-sterile 5 produced no sclerotium-like bodies, but formed abundant endoconidia, and grew more slowly than self-sterile 1. It was found as a single variant in the ascospore progeny of a self-fertile 1 isolate.

Self-sterile 6, similar to self-sterile 5, also produced no sclerotium-like bodies. It was somewhat darker and produced few endoconidia. It occurred as a recombination type from the cross, self-sterile 5 × self-sterile 1.

MATERIALS AND METHODS

The cultures of *Ceratostomella fimbriata* on which the present investigations were based were isolated from black-rot lesions on sweet potatoes or

were obtained from cooperators in other Southern states. The medium ordinarily used for culturing this fungus and for comparing cultural types was potato-dextrose agar.[2] Special media described later were used in a few experiments. Single spores, isolated with a micro-manipulator, were germinated on hanging drops of agar suspended over glass rings. Very few single spores germinated on freshly made agar drops. It was, however, found that when the hanging drops of agar were kept for three days over a water-saturated filter paper before single spores were isolated, the germination was raised from approximately 10 to 50–90 per cent. After the single spores germinated, the hanging drops were transferred to agar slants in test tubes. After 2 weeks the cultures were classified as to cultural type. Two methods were used to combine or to cross nonperithecial isolates with isolates of other cultural types. One method was to allow isolates to grow together. The second method was a "spermatizing" technique, where spores from the test isolate were added to week-old cultures of nonperithecial isolates. The second method made crosses possible between self-sterile isolates and self-fertile isolates. Certain self-sterile isolates "spermatized" in this manner formed long-necked perithecia within 3 days in contrast to the 7 or more days required for perithecium formation when self-fertile isolates were grown alone.

ASCOSPORE ISOLATIONS FROM SELF-FERTILE STRAINS

When single ascospores were isolated, it was observed that nonperithecial (self-sterile) as well as perithecial (self-fertile) cultures were se-

TABLE 1.—*Results of single ascospore isolations from 4 self-fertile strains of Ceratostomella fimbriata*

Parental strain	No. cultures tested[a]	Number of isolates of each strain in ascospore progenies		
		Cultural strains	No. cultures	Total
Self-fertile 1	103	Self-fertile 1 (Parent)	942	
		Self-sterile 1	634	
		Self-sterile 5	1	1377
Self-fertile 2	6	Self-fertile 2 (Parent)	268	
		Self-sterile 2	151	419
Self-fertile 3	2	Self-fertile 3 (Parent)	23	
		Self-sterile 3	23	46
Self-fertile 4	11	Self-fertile 4 (Parent)	42	
		Self-sterile 4	43	85
TOTAL				2127

a Two strains were obtained from every culture.

2 Formula of potato-dextrose agar used to make one liter:
Water extract from 200 gm. of peeled potatoes
10 gm. dextrose
20 gm. agar-agar
Water to make 1000 cc.
Sterilize one hour at 15 lb. pressure

cured. To determine the ratio in which the two strains occurred, asco-
spores were isolated from 103 cultures of the self-fertile 1 strain and from
a lesser number of three other self-fertile strains. The results are given
in table 1.

From each culture of the four self-fertile strains tested, self-sterile
strains were obtained in the ascospore progeny. A 3:2 ratio of parental
strains to the variant strains was obtained in ascospore isolations from
the self-fertile 1 strain.

To determine whether or not both self-fertile and self-sterile strains
occurred in the same perithecium, ascospores were isolated from individual
perithecia. The 22 individual perithecia studied included 11 of the self-
fertile 1 strain, 2 of the self-fertile 2 strain, and 9 of the self-fertile 4
strain. From each of these 22 perithecia the self-fertile parental strain
and its self-sterile variant strain were isolated.

A number of self-fertile 1 cultures were obtained from black-rot lesions
on sweet potatoes from 7 states other than Louisiana, and isolations from
single ascospores were made. The results of these isolations, given in

TABLE 2.—*Occurrence of self sterile strains in ascospore progenies of self fertile strains of Ceratostomella fimbriata from 8 states*

| Source of isolate | Number of single ascospore isolates of each cultural type | | |
	Self fertile 1	Self-sterile 1	Total
Alabama	15	8	23
Georgia	6	1	7
Louisiana	190	137	327
Mississippi	6	1	7
North Carolina	111	55	166
Tennessee	17	14	31
Virginia	10	6	16
West Virginia	53	33	86
TOTALS	408	255	663
Per cent	61 5	38.5	100.0

TABLE 3.—*Strains isolated from cultures of the self fertile 1 strain in successive generations*

Generation	No. cultures tested	Self-fertile 1 (parent)	Self-sterile 1 (variant)	Total
First	1	12	4	16
Second	1	8	5	13
Third	1	4	1	5
Fourth	1	3	2	5
First	1	31	17	48
Second	16	107	79	186
Third	1	38	30	68
First	1	56	17	73
Second	1	41	19	60
TOTALS	24	300	174	474

table 2, show that both the self-fertile 1 and self-sterile 1 strains were obtained in the ascospore progenies from every culture.

The occurrence of cultures of the self-sterile 1 strain in the ascospore progenies of self-fertile 1 strains continued for four ascospore generations (Table 3).

CONIDIAL ISOLATIONS

The large number of cultures of self-sterile strains appearing among the single ascospore cultures of self-fertile strains made it seem advisable to determine what strains might appear among the cultures from endoconidia. Isolations of single endoconidia were made from 7 of the strains.
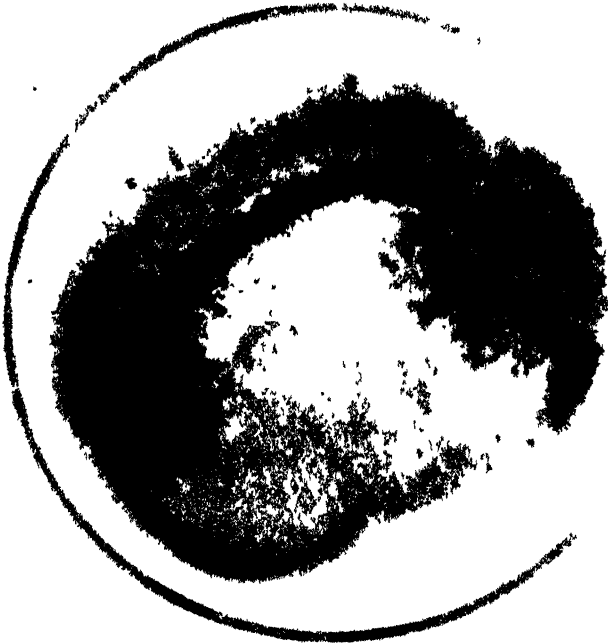
Fig. 2. Perithecia at line of contact between self fertile 1 and self sterile 1 strains of *Ceratostomella fimbriata*.

Of the 581 conidial cultures isolated and compared, all were of the respective parental strain. These included 293 from the self-fertile strains 1, 2, and 4, and 288 from the self-sterile strains 1, 2, 4, and 5.

When endoconidia were stained they were found to be uninucleate. This indicated that the mycelium of these strains was not heterocaryotic in the manner reported by Dodge (10) in *Neurospora tetrasperma*.

COMPATIBILITY OF CULTURES

When cultures of the two original strains (self-fertile 1 and self-sterile 1) isolated from black-rot lesions were paired, a line of perithecia formed where they grew together (Fig. 2). A line of perithecia also formed where isolates of self-fertile 4 and self-sterile 1 grew together.

When a water suspension of endoconidia from the self-fertile 4 strain was placed on the mycelium of the self-sterile 1 strain, fertile long-necked perithecia appeared at that spot within 3 days (Fig. 3). No perithecia
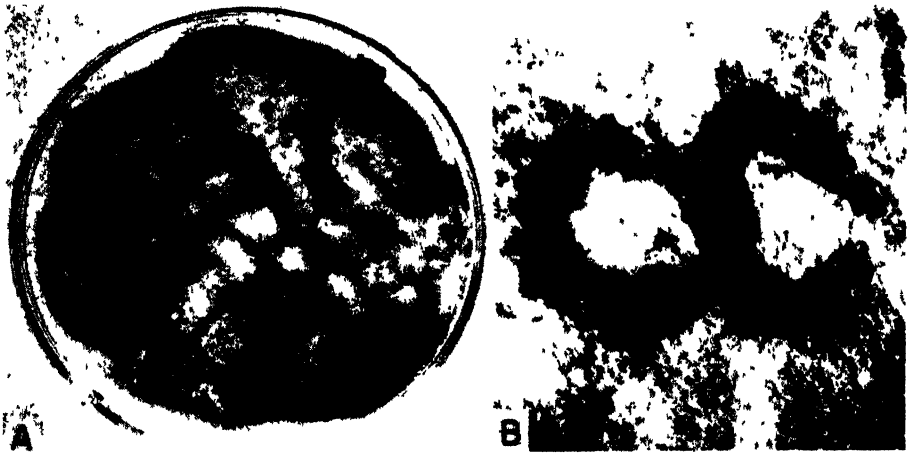
FIG. 3. A. Perithecia formed where spore suspension of self-fertile 4 strain was placed on mycelium of self-sterile 1 strain of *Ceratostomella fimbriata*. B. Enlarged view showing long-necked perithecia.

formed where only a drop of water was placed on the mycelium of the self-sterile 1 strain. When the reverse cross was made, *i.e.*, when a water suspension of endoconidia from the self-sterile 1 strain was placed on the mycelium of the self-fertile 4 strain, no long-necked perithecia appeared; the self-fertile 4 strain, which produced short-necked perithecia, thus did not produce the long-necked perithecia which formed on the line between the self-fertile 4 and self-sterile 1 strains. When a suspension of self-fertile 1 or 2 endoconidia was placed on self-sterile 1 or 2, perithecia also were formed.

These results indicated that there are sex or compatibility groups in this species, and that the self-sterile 1 or 2 strains functioned as a female, forming long-necked perithecia in combination with self-fertile isolates.

The ability of 8 of the 10 strains to form perithecia in various combinations was studied. Such combinations were made either by growing two strains in the same Petri plate or by "spermatizing" one strain by the other. Combinations which were not tested are indicated by a question mark in table 4, where the results are summarized.

The self-sterile strains 1 and 2 produced long-necked perithecia in combination with self-fertile strains 1, 2, and 4. Self-sterile 1 and self-sterile 5, as well as self-sterile 2 and self-sterile 5, produced long-necked perithecia in combination. When endoconidia from one strain were placed on the mycelium of the other strain, self-sterile strains 1 and 2 again functioned as females, and self-sterile 5 functioned as a male, since perithecia would form on self-sterile 1 and 2, but not on self-sterile 5.

TABLE 4.—*The results of combining cultures of 8 strains of Ceratostomella fimbriata*

Strain	Self-fert. 1	Self-fert. 2	Self-fert. 4	Self ster. 1	Self-ster. 2	Self-ster. 4	Self-ster. 5	Self-ster. 6
Self-fertile 1	−	−	−	+	+	−	−	−
Self-fertile 2	−	−	−	+	+	−	−	?
Self fertile 4	−	?	−	+	+	−	−	−
Self sterile 1	+	+	+	−	−	−	+	−
Self-sterile 2	+	+	+	−	−	−	+	−
Self-sterile 4	−	−	−	−	−	−	−	−
Self sterile 5	−	?	−	+	+	−	−	−
Self-sterile 6	−	−	−	−	−	−	−	−

+ = perithecia formed.   − = no perithecia formed.   ? = combination not tried.

INHERITANCE OF CHARACTERS AFFECTING SEX AND CULTURAL CHARACTERISTICS

The occurrence of perithecia where certain of the strains were paired offered an opportunity to determine whether or not fertilization occurred, and also to study the inheritance of some of the genetic factors determining the differences among the various strains.

Table 5 gives the results of ascospore isolations from 5 pairings between self-fertile and self-sterile strains. The genotypes of the various strains on the basis of the results are given. In the first pairing, between self-fertile 1 and its variant self-sterile 1, the ascospore progeny included 307 of the self-fertile parent, 297 of the self-sterile parent, and 37 cultures of self-fertile 4 strain. These 37 cultures were probably mutants, since the back-cross, self-fertile 4 × self-sterile 1, gave four strains in approximately

TABLE 5.—*Results of ascospore isolations from 5 compatible combinations between self-fertile and self sterile strains of Ceratostomella fimbriata*

Parental strains	Parental genotypes	Progeny strains and genotypes							Total
		SF 1 ABC	SF 2 ABc	SF 4 A₁BC	SS 1 AbC	SS 2 Abc	SS 4 A₁bC	Other strain	
Self-fertile 1 × Self-sterile 1	ABC × AbC	307		37[a]	297				641
Self-fertile 2 × Self-sterile 1	ABc × AbC	36[b]	28		32	9[c]		23[d]	128
Self-fertile 2 × Self-sterile 2	ABc × Abc		7			6			13
Self-fertile 1 × Self-sterile 2	ABC × Abc	20[b]	16		16	5[c]			57
Self-fertile 4 × Self-sterile 1	A₁BC × AbC	176	1[d]	195	158		155		685
								TOTAL	1524

[a] These are considered variants which are not recombinations from the cross of the parental isolates.
[b] It seemed that this group could be divided into two minor sub-groups, but sufficient studies were not made to be positive of separating them for genetic studies.
[c] It is not known why so few of this group occurred.
[d] Insufficient information to classify.

a 1:1:1:1 ratio in 685 cultures, as well as one culture of another strain, self-fertile 2. The isolations from the first pairing, self-fertile 1 × self-sterile 1, would not prove that fertilization between the two strains took place, since ascospores from self-fertile 1 cultures growing separately also gave these strains in a 3:2 ratio. The crosses self-fertile 2 × self-sterile 1 and self-fertile 1 × self-sterile 2 indicated that a fertilization had taken place in these combinations since four strains occurred in the progeny of each cross. It is therefore presumed that fertilization also took place in the cross self-fertile 1 × self-sterile 1.

On the basis of these results, it is considered that these self-sterile variants differ from their self-fertile parents by one factor at locus B, and that self-fertile strains 1 and 4 have different genes at locus A. Self-fertile 2 strain differed from self-fertile 1 at a third locus, C.

Ascospore isolations also were made from the perithecia formed where certain self-sterile strains came together. The results are given in table 6,

TABLE 6 —*Results of isolations of crosses between self sterile strains of Ceratostomella fimbriata*

Parental strains	Parental genotypes	SF 1 ABC	SS 1 AbC	SS 5 aBC	SS 6 abC	SF 2 ABc	SS 2 Abc	Total
Self sterile 1 × Self sterile 5	AbC × aBC	113	101	116	84			414
Self sterile 2 × Self sterile 5	Abc × aBC	3	3	6		9	7	28
						TOTAL		442

together with the postulated genotypes. From one cross, self-sterile 1 × self-sterile 5, 414 cultures were isolated and classified. Four strains occurred in the progeny in approximately a 1:1:1:1 ratio These strains included the two parental strains, the self-fertile 1 strain from which the two parental strains were obtained, and the recombination strain expected where the parents differed by two factors. This strain, self-sterile 6, as shown in table 4, was not compatible with the other strains. Self-sterile 2 also was paired with self-sterile 5. Only 28 cultures were isolated from this cross, but 5 of the 8 strains expected were obtained, and, of these, 12 were self-fertile isolates.

These results indicate that there are at least two loci controlling the production of perithecia in this fungus. The pairing of two self-sterile strains, each containing only one of the two genes necessary for perithecium production, results in the formation of fertile perithecia. The recombination of the genes necessary for perithecium production reconstitutes the homothallic or self-fertile strain.

EFFECT OF THIAMIN ON PERITHECIUM PRODUCTION

Barnett and Lilly (4) demonstrated that thiamin was necessary for the production of perithecia by *Ceratostomella fimbriata*. The isolates

which they studied would be the same as the self-fertile 1 strain reported in this paper. In the present study, isolates of various strains were grown on Czapek's and on potato-dextrose agar, with and without the addition of thiamin. Self-fertile 1 or self-fertile 4 isolates did not produce perithecia unless 5 μg. of thiamin was added to the media. However, the addition of 100 μg. of thiamin to Czapek's media or to potato-dextrose agar did not induce the production of long-necked perithecia by self-fertile 4 isolates, which produced short-necked perithecia on potato-dextrose agar. Additions of 100 μg. of thiamin to the same two media did not induce the production of perithecia by isolates of the self-sterile 1, self-sterile 3, or self-sterile 5 strains. The other cultural types were not tested.

Thus, while the appearance of some of these strains can be simulated by thiamin-deficiency, these strains, when grown on potato-dextrose agar, apparently represent distinct genetic entities rather than thiamin-starved isolates.

PATHOGENICITY OF STRAINS

The pathogenicity of 9 strains was determined by inoculation tests. The inoculations were made by inserting conidia or mycelium into wounds in the surface of sweet potatoes. After inoculation, the sweet potatoes were placed in paper sacks and examined for lesions after 7 or more days had passed. The comparisons of pathogenicity were made on the basis of the area of the black-rot lesions and the depth of penetration.

One hundred and seventy-three cultures representing 9 strains were tested for pathogenicity. Self-fertile strains 1, 2, 3, and 4 produced typical lesions, although those caused by self-fertile 2 were smaller. Self-sterile strains 1, 2, 3, and 4 were also pathogenic, although self-sterile 2, like its parental strain self-fertile 2, also produced small lesions. The strain self-sterile 5 was nonpathogenic.

Forty-six cultures from the cross self-sterile 1 (pathogenic) × self-sterile 5 (nonpathogenic), were tested for pathogenicity to sweet potatoes. These 46 cultures included the 4 strains which were obtained from this cross. The results (table 7) show that pathogenicity and nonpathogenicity were inherited in a 1:1 ratio (Fig. 4). This indicated that the self-sterile 5 strain lost its pathogenicity when it arose from the parental strain,

TABLE 7.—*Pathogenicity to sweet potato of ascospore progeny from the cross between the two strains, self sterile 1 × self-sterile 5, of Ceratostomella fimbriata*

Cross	Cultural type of the progeny	Genotype	No. of isolates tested	Pathogenicity
Self-sterile 1 (AbC) × Self-sterile 5 (aBC)	Self-fertile 1	ABC	11	Pathogenic
	Self-sterile 1	AbC	13	Pathogenic
	Self-sterile 5	aBC	15	Nonpathogenic
	Self-sterile 6	abC	7	Nonpathogenic
	TOTAL		46	

self-fertile 1, and that the pathogenicity factor in question was apparently
located at the A locus.

FIG. 4. Surface view and cross section of sweet potatoes inoculated with 4 strains
of *Ceratostomella fimbriata* derived from a cross between the self-sterile 1 and self-sterile
5 strains. The two potatoes on left inoculated with pathogenic, the two on right with
nonpathogenic strains.

DISCUSSION

The continued occurrence in *Ceratostomella fimbriata* of a large number
of self-sterile strains in ascospore cultures of self-fertile strains is similar
in many respects to the phenomenon reported by Lindegren (17) in
Neurospora and in *Glomerella* by Andes (1) and Lucas, Chilton, and
Edgerton (19). Other species of *Ceratostomella*, as *C. ips.* (16), also give
self-sterile strains from ascospores.

Of the mechanisms which could be responsible for the results with the self-fertile strains, segregation and mutation appear to be the most likely ones. For segregation to occur, the fusion nucleus in the ascus must be heterozygous. Since ascospores are apparently uninucleate and the nuclei present in them are a result of meiosis, it would appear that the nuclei that fuse in the ascus in the next generation should be alike. It is possible that mutation would give genetically different nuclei which would fuse in the ascus and result in the segregation for parental self-fertile strains and self-sterile ones. If mutation is responsible, a selection of variant nuclei probably occurs in the formation of perithecia and ascospores, otherwise strains should have been found among the endoconidial isolates. If no mutation occurs, segregation implies that there are two chromosomes in the nucleus of the self-fertile strain which are capable of pairing at meiosis but which carry different genes at the same locus. The fusion nucleus in the ascus would then carry more than two chromosomes which were homologous. This would necessitate the adoption of the concept that the haploid stage of the fungus was 2n and the diploid 4n. The ratios from the crosses made among the various strains were those expected from the crosses of haploid rather than diploid isolates. It would also be necessary to account for the high occurrence of the self-sterile 1 strain and very low occurrence of the self-sterile 5 strain from the self-fertile 1 strain.

The nature of the differences between homothallic and heterothallic fungi is an interesting problem. In *Ceratostomella fimbriata* it would seem that strains are homothallic when they possess in the same nucleus two main factors which govern the initiation and formation of perithecia and ascospores. If one of these factors is absent the strain is self-sterile and if both are absent the strain becomes neuter. If the two factors are present in nuclei in different thalli the cultures behave as heterothallic ones and when brought together form perithecia and ascospores.

SUMMARY

1. Ten strains of *Ceratostomella fimbriata*, differing in pathogenicity, morphology, and compatibility were isolated and described. These strains were obtained from the ascospore progeny of cultures isolated from black-rot lesions on sweet potato or from the crosses of variants of the same origin.

2. The ascospore progeny of 4 self-fertile strains included not only the parental self-fertile strains but its respective self-sterile strain. Single conidium isolates from single-spore cultures were of the parental type.

3. Long-necked perithecia formed on the line between self-fertile and certain self-sterile strains, as well as between certain self-sterile strains. Isolates of still other strains did not produce perithecia alone or in combination with other self-sterile strains.

4. The ascospore progeny from perithecia formed by combinations of strains included the parental types alone, or both parental and recom-

bination types. The results of these crosses suggest that a combination of at least two independently inherited genes are necessary for perithecium production.

5. Ascospore isolations from a homothallic strain gave rise to heterothallic strains. The progeny of the cross of two heterothallic strains included isolates apparently identical to the original homothallic strain.

6. Half of the progeny of the cross of pathogenic × nonpathogenic isolates were apparently nonpathogenic to sweet potato.

7. The addition of thiamin to Czapek's medium was necessary for perithecium formation by isolates capable of forming perithecia on potato-dextrose agar. The addition of thiamin to potato-dextrose agar did not induce the formation of perithecia by isolates which produced no perithecia upon potato-dextrose agar.

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REACTIONS OF THREE SPECIES OF DODDER TO HOST PLANTS USED IN VIRUS DISEASE INVESTIGATIONS

C . F . L A C K E Y

Work by Bennett[1, 2] showed that some dodders were useful in the transmission of various viruses to different host plants. Following this work many investigators have used dodder in the transmission of viruses to a wide variety of host-plants. Although many of the dodders have an extensive host range, their usefulness in virus transmission is limited by the extent of this range. Studies were undertaken on three species of dodder to determine how well they would parasitize certain varieties of host plants. A brief report of this work has been given[3].

MATERIALS AND METHODS

The two species of dodder which were used in Bennett's[1] first studies were used in these experiments. These are the relatively large-stemmed one, *Cuscuta subinclusa* Dur. and Hilg., and the very small-stemmed species, *C. californica* Choisy, both of which grow vigorously on healthy sugar beet petioles. The host plants used were those most commonly used in the virus work at this laboratory,—sugar beet, Turkish tobacco (*Nicotiana tabacum* L. var. Turkish), tree tobacco (*N. glauca* Graham), tomato, and two varieties of cucurbits, cucumber and Zucchini squash. *C. americana* L., a dodder with still larger stems than either of the other two, has recently been added to the list of dodders tested by Bennett[4]. It was, therefore, studied anatomically also by the writer on sugar beet, tomato, and tobacco.

The study of the reaction of the three species of dodder on different host plants was made from free-hand sections of fresh tissues and from material fixed in alcohol-formalin-acetic solution. The sections of the fixed material were cut 20 μ thick. These preparations were stained in dilute iron-haematoxylin without destaining, and counter-stained with dilute safranin. Sometimes light green in clove oil was used with these two stains or as a counter-stain with the safranin alone.

EXPERIMENTAL RESULTS

Sugar beet and tobacco are good hosts for all the dodder species studied. The dodders grow luxuriantly on these host plants without any noticeable effect on the tissues through which the haustoria pass. Figure 1, A shows

[1] Bennett, C. W. Acquisition and transmission of viruses by dodder (*Cuscuta subinclusa*). (Abstr.) Phytopath. 30: 2. 1940.
[2] ————. Studies of dodder transmission of plant viruses. Phytopath. 34: 905–982. 1944.
[3] Lackey, C. F. Tissue relationships of certain dodders to some host plants used in virus disease studies. (Abstr.) Phytopath. 37: 362. 1947.
[4] Bennett, C. W. Unpublished data.

Cuscuta subinclusa on a beet petiole. Haustorial hyphae precede the main body of the haustorium. The xylem vessels of this dodder develop and fuse with those of the host plant when the haustorium has reached the bundle, as in the blending of tissues in a successful graft. This is shown in figure
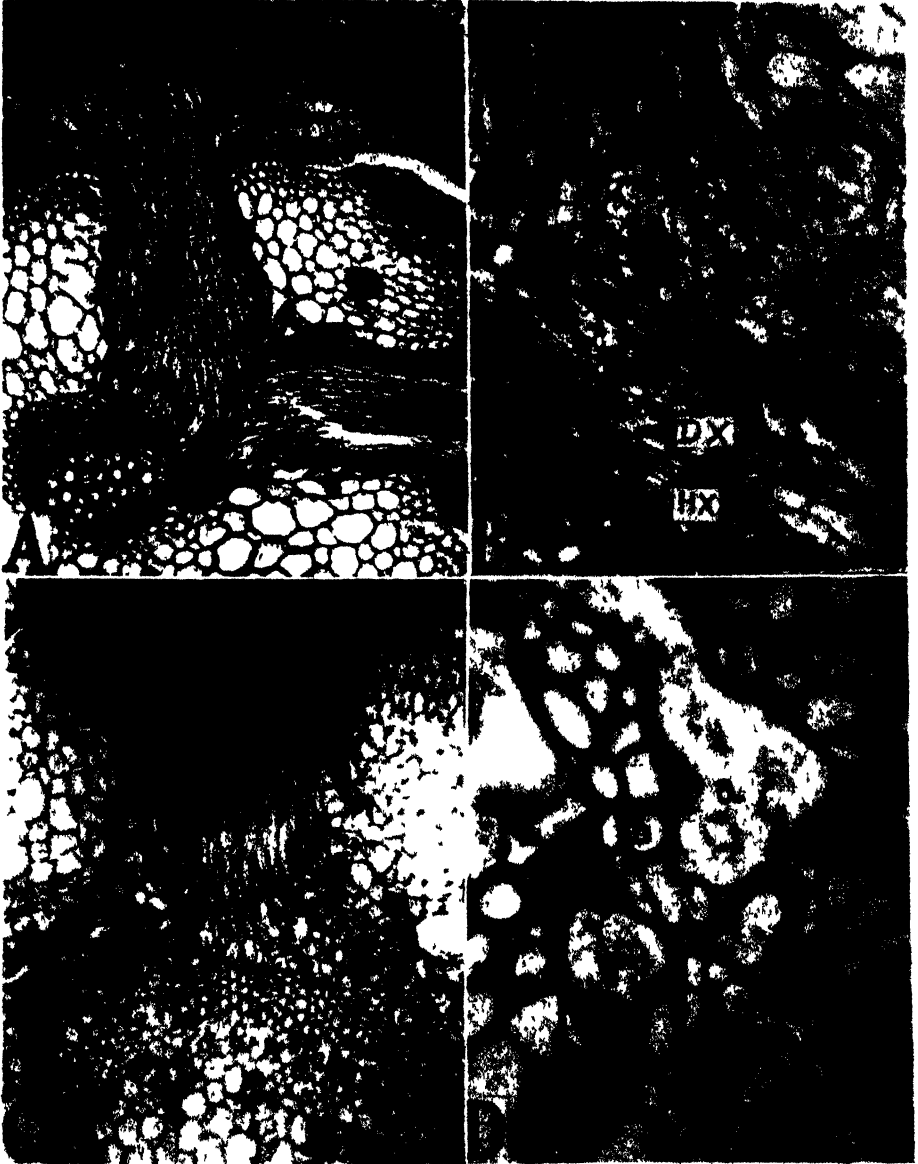
FIG. 1. Dodder haustoria in beet and tobacco. A Two haustoria of *Cuscuta subinclusa* contacting vascular bundle of sugar beet petiole. B Longitudinal section of beet petiole showing how xylem vessels of dodder haustorium (dx) blend with xylem of host plant (hx). C. Same dodder on *Nicotiana tabacum* with haustorial hyphae (b) of haustorium (a) contacting external phloem. D. Haustorial hypha (a) which has penetrated beyond xylem (b) and is growing in direction of internal phloem of tobacco stem.

1, B. The other dodders developed in the same way on the sugar beet.

On the stem of tobacco the growth of dodder is somewhat different. In tobacco the vascular bundles form a distinct ring between cortex and pith. There are groups of phloem cells forming an external and an internal phloem on each side of the ring. A dodder haustorium sends its hyphae along the outer edge of this vascular ring making contact with the external phloem tissue first as shown in figure 1, C. After this contact by the hyphae the xylem vessels usually develop and contact the xylem tissues of the host plant. The haustorial hyphae sometimes grow on through the ring of xylem vessels and xylem parenchyma toward the internal phloem as in figure 1, D.
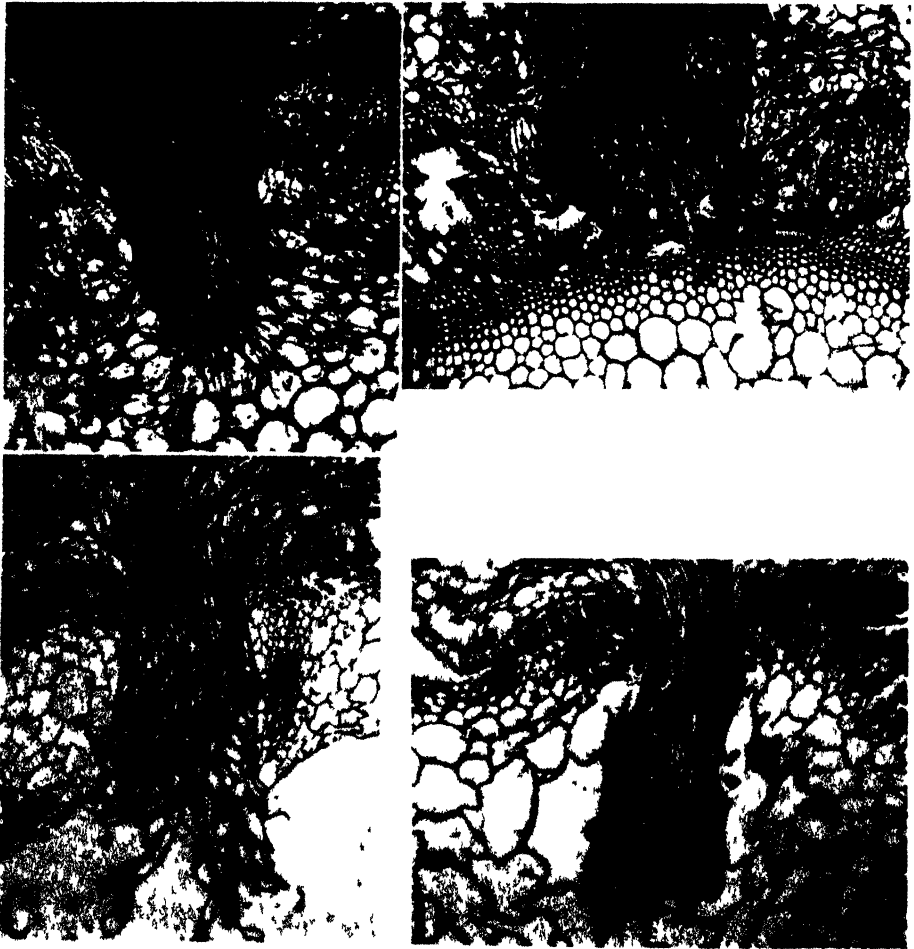
FIG. 2. Dodder on tobaccos and cucurbits. A. *Cuscuta americana* haustorium on *Nicotiana tabacum*, having penetrated beyond xylem ring and external phloem where hyphae make only limited contact with internal phloem. B. *C. americana* on *Nicotiana glauca*; haustorium has failed to pass through xylem ring (x), and hyphae (h) have made good contact with external phloem. C. *C. subinclusa*, having grown through stem of cucumber into hollow center making very little vascular contact. D. Haustorium of *C. subinclusa* stranded among large cells of Zucchini squash stem, making no vascular contacts.

Cuscuta americana grows more luxuriantly on tree tobacco than on Turkish tobacco. A study of the host tissues of these varieties of tobacco parasitized by *C. americana* suggests a possible explanation. It was observed that the haustorium and its hyphae in Turkish tobacco grew past the external phloem and contacted only a limited amount of internal phloem (Fig. 2, A), while on tree tobacco growth of the haustorium was confined to the bark where the greatest amount of phloem tissue exists (Fig. 2, B).

Two species of cucurbits were tested. The first was cucumber (variety Long Green) and the other, Zucchini squash. The cucumber has thin, hol-
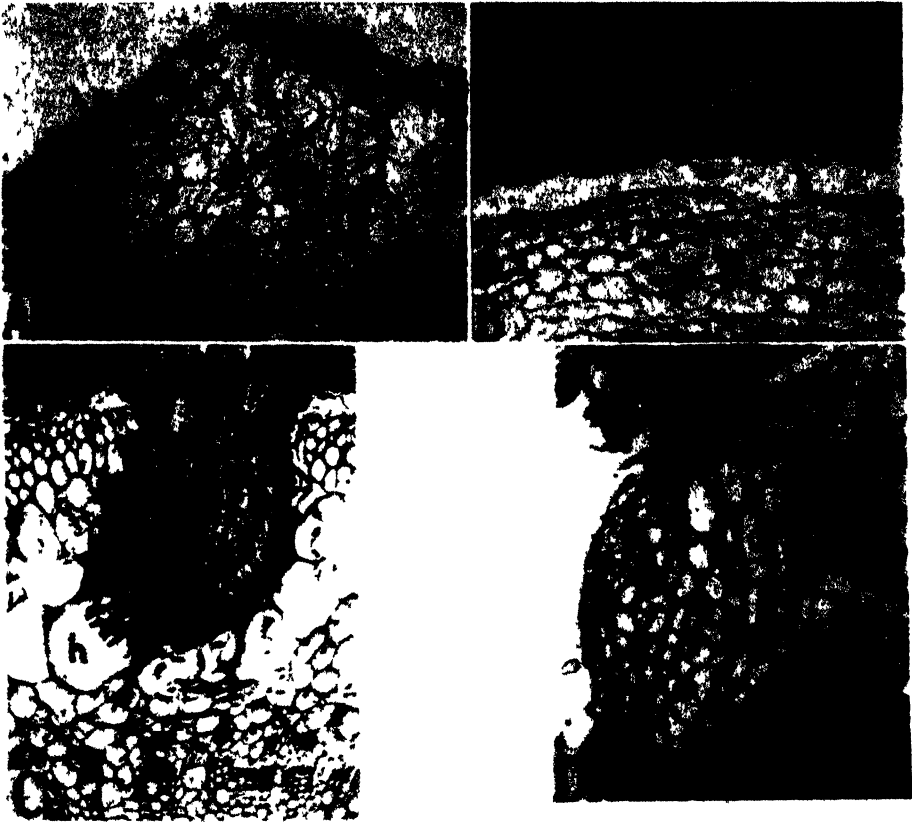
FIG. 3. A. Transverse section of tomato stem which has been lightly contacted (a) by dodder haustorium, producing severe hypertrophy and disorganization of cortex cells (cc); normal cells at (nc). B Transverse section of tomato stem (t), and dodder stem (d) which has encircled tomato stem for 6 weeks while growing on tobacco plant; haustorial initials (hi) have failed to develop into haustoria in any part of the encircling dodder stem. C. *Cuscuta americana* on tomato stem; haustorium has caused some hypertrophy of cell (h) but not sufficient to prevent some vascular contact D. *C. californica* on tomato stem, making a vigorous growth with no hypertrophy or abnormal reaction of host cell and haustorial hyphae (c).

low stems and relatively small cells *Cuscuta subinclusa* grew for a while on this species but not vigorously. In a majority of cases, as shown in figure 2, C, the haustoria grew right through the stem into the hollow center, with few vascular contacts. In other cases the haustoria grew di-

rectly to the vascular bundles. Zucchini squash has a large stem with big cells and a large hollow center. *C. subinclusa* will not grow on this plant without being connected to a favorable host. A tobacco plant with a vigorous growth of *C. subinclusa* on it was placed among the stems of a Zucchini squash and the fast-growing stems of the dodder twined around the stems of the squash leaves. The dodder was allowed to grow for 4 or 5 weeks until it seemed well established on the squash stems. When the dodder was cut loose from the tobacco plant and left on the squash plant it began to decline rapidly. Microscopic examinations made of these squash stems with dodder on them showed (Fig. 2, D) that the haustorium had developed and had grown into the host stem as deeply as it does in other host stems. The squash cells surrounding the haustorium were abnormally large and apparently devoid of contents. The haustorium appeared to be stranded among them and unable to make contact with a vascular bundle for nourishment.

Cuscuta americana will live for a considerable time on the tomato but does not grow luxuriantly on it. It induces some hypertrophy of cortex cells (Fig. 3, C). The haustoria do not make as good contact with the vascular tissues of tomato as they do with tissues of beet petioles. Some haustorial hyphae apparently are isolated by the large cells.

Cuscuta californica will grow well for long periods on the stem of the tomato plant. This dodder also attaches itself to the petiole of a compound leaf where it grows much more luxuriantly than on the stem. The relationship between this small dodder and the tomato stem is similar to that between *C. subinclusa* and the tobacco stem or beet petiole. There apparently is no abnormal cell growth produced by the contact of dodder haustorium and the stem of the host plant, as is shown in figure 3, D. In this figure, the haustorial hyphae are unusually long. The usual growth of the haustoria of this dodder in the tomato stem is similar to its growth on beet and tobacco, with one exception. The haustoria do not penetrate very far into the tomato stem before long hyphae are sent out to the vascular tissue. The haustorium shown in figure 3, D has not penetrated much beyond the epidermal layer of cells. On beet or tobacco the haustoria penetrate the cortex much deeper before their hyphae reach the vascular ring.

The reaction between *Cuscuta subinclusa* and the tomato plant is the most unusual of all host reactions observed. A mutual antagonism seems to exist between the host and the parasite, resulting in an abnormal development of the haustorium as it comes in contact with the tomato stem. No penetration takes place, but this contact induces an extremely abnormal growth in the cortex parenchyma of the tomato. Figure 3, A, a transverse section of tomato stem so contacted, shows most cells extremely hypertrophied and disorganized, with a few normal cells at the left side. Figure 3, B shows the effect of mere contact between a haustorium and an old tomato stem. *C. subinclusa*, while growing vigorously on a tobacco plant, was trained on a mature tomato plant stem. The dodder completely en-

circled the tomato stem and grew for 6 weeks while still attached to the original host. When cut loose from the tobacco plant, the dodder declined rapidly and soon died. Sections were made of the tomato stem with the encircling dodder stem. On no part of the encircling dodder were haustoria produced. It is evident from figure 3, B that the contact stimulus started the development of haustorial initials, but they never developed beyond a rudimentary stage. The tomato stem being fully mature at the time the dodder was attached, no hypertrophy developed in the cortex of the stem. This indicates an inhibitory effect of the tomato stem on haustorial development, since this dodder produces fully developed haustoria even on contact with a wooden stick.

SUMMARY AND CONCLUSIONS

Studies were made on the reactions of three species of dodder (*Cuscuta subinclusa* Dur. and Hilg., *C. californica* Choisy, and *C. americana* L.) on the following host plants commonly used in virus disease research: sugar beet, tobacco, tomato, and two varieties of cucurbits (cucumber and Zucchini squash).

These three dodders grow well on sugar beet and tobacco. They do not grow vigorously or for very long on cucumber. *Cuscuta subinclusa* will establish itself on Zucchini squash but will not live independently on it. The haustoria fail to cross the barrier of large cells in the squash stem to contact vascular tissue.

Cuscuta americana will grow for a short period on tomato but not vigorously. The haustoria induce some cell hypertrophy in the tomato stem and that, to some degree, seems to prevent haustorial hyphae from contacting vascular tissue.

Cuscuta subinclusa will not establish itself on the tomato stem but mere contact of the haustoria with the stem may produce severe hypertrophy and abnormal development of the cortex cells. This dodder growing on a tobacco plant was allowed to encircle the main stem of a mature tomato plant. Sections made of the dodder on the tomato stem showed haustorial initials which never developed beyond the rudimentary stage. Since haustoria are developed upon contact even with a wooden stick, this indicates an inhibitory effect of the tomato stem on haustorial development.

Proper selection of the species of dodder and the host plant is necessary if one is to succeed in transmitting virus by dodder in any given case.

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INCIDENCE OF FUSARIUM INFECTION AS AFFECTED BY ROOT-KNOT NEMATODES

W . D . M c C l e l l a n a n d J . R . C h r i s t i e [1]

There are many statements in the literature to the effect that the severity of Fusarium wilt is increased by root-knot nematodes, although good supporting experimental evidence has been lacking. Indirect evidence has been obtained, however, indicating that when soil fumigants are used to control nematodes the severity of cotton wilt is reduced[2,3]. Both *Heterodera marioni* (Cornu) Goodey and *Fusarium oxysporum* f. *gladioli* (Massey) Snyder and Hansen were encountered on gladiolus in a soil-fumigation experiment in the Rio Grande Valley of Texas, which raised the question concerning the effect of root-knot on the incidence of Fusarium infection. Consequently, experiments were set up at the Plant Industry Station, Beltsville, Maryland, to determine whether or not root-knot nematodes increase the incidence of Fusarium infection in gladiolus, in tomato, and in squash. The Fusaria used were vascular pathogens in gladiolus and in tomato, and a cortical pathogen in squash.

For these tests the plants were grown in steamed 8-in. clay pots filled with autoclaved soil consisting of a mixture of one part sand, one part muck, and one part compost. Four hundred pots were used in experiment 1, with 20 pots in each of 20 treatments. The different inoculation treatments are listed in table 1. In addition to those listed for Picardy gladiolus, there were odd-numbered companion treatments consisting of 20 pots of noninoculated Picardy gladiolus, 20 inoculated with population 15 of *Heterodera marioni*, and 20 inoculated with a mixture of populations 3B and 15 of *H. marioni* to serve as controls. Similar companion treatments were used with Dr. F. E. Bennett gladiolus, tomato, and squash in both experiments 1 and 2. Inocula were prepared as follows: the Fusaria were grown on sterile wheat, ground in a food chopper, and 2 qt. of this mixed with 30 qt. of soil; the nematodes were increased in the greenhouse on squash roots, which were then ground and mixed with soil. The *Fusarium oxysporum* f. *gladioli* used was a mixture of our isolates 106, 123, and 127 obtained from gladiolus corms; the *F. oxysporum* f. *lycopersici*

[2] Smith, A. L. Control of cotton wilt and nematodes with a soil fumigant. Phytopath. 38: 943–947. 1948.

[3] Taylor, A. L., H. D. Barker, and P. H. Kime. Further observation on the nematode-Fusarium-wilt experiment at Lumberton, North Carolina. (Abstr.) Phytopath. 30: 710. 1940.

(Sacc.) Snyder and Hansen was F. L. Wellman's R5-6; and the *F. solani* f. *cucurbitae* Snyder and Hansen was obtained from W. C. Snyder. Two

TABLE 1.—*Effect of root-knot nematodes on the incidence of fusarium infection in gladiolus, tomato, and squash*

Treatment number[a]	Plants inoculated with	Relative root-knot index[b]	Number of plants infected with Fusarium[c]	Average number of days prior to harvest when symptoms appeared[d]
	Experiment 1			
	Gladiolus var. Picardy			
2	*Fusarium oxysporum* f. *gladioli*		60	19.0
4	*Fusarium oxysporum* f. *gladioli* plus *Heterodera marioni* 15		60	25.3
6	*F. oxysporum* f. *gladioli* plus *H. marioni* 15 and 3B		57	20.7
	Gladiolus var. Dr F. E. Bennett			
8	*F. oxysporum* f. *gladioli*		56	44.6
10	*F. oxysporum* f. *gladioli* plus *H. marioni* 15		49	47.6
12	*F. oxysporum* f. *gladioli* plus *H. marioni* 15 and 3B		55	50.5
	Tomato var. Marglobe			
14	*F. oxysporum* f. *lycopersici*		14	18.3
16	*F. oxysporum* f. *lycopersici* plus *H. marioni* 3B	3.5	12	21.3
	Squash var. White Bush			
18	*F. solani* f. *cucurbitae*		20	37.7
20	*F. solani* f. *cucurbitae* plus *H. marioni* 3B	4.0	20	43.6
	Experiment 2			
	Squash var. White Bush			
22	*F. solani* f. *cucurbitae*		13	17.4
24	*F. solani* f. *cucurbitae* plus *H. marioni* 5	3.7	9	13.1
26	*F. solani* f. *cucurbitae* plus *H. marioni* 7	3.9	12	10.8
28	*F. solani* f. *cucurbitae* plus *H. marioni* 8	4.0	13	11.0
30	*F. solani* f. *cucurbitae* plus *H. marioni* 15	3.7	14	16.3

[a] The odd-numbered treatments are the controls and are not listed. See text.

[b] 0.0 indicates no galling and 4.0 indicates maximum galling. This index was obtained on companion plants inoculated with nematodes only.

[c] Sixty plants were inoculated in each of treatments 2 to 12, 20 in each of treatments 14 to 20, and 16 plants in each of treatments 22 to 30.

[d] This indicates the rapidity with which infection took place; i.e., small numbers indicate that the incidence of infection was slower. The addition of root-knot nematodes did not result in a significant increase in the incidence of the disease except in treatments 4 and 12. These increases are significant at the 5 per cent level only.

populations of *H. marioni* were used, 3B and 15. Population 3B was obtained from potatoes from Harlingen, Texas, and population 15 was obtained from gladiolus from Linn, Texas. The soil in the pots was infested

as follows: 1 qt. of the autoclaved soil was placed in the bottom of each pot and this was covered with either 250 ml. of the Fusarium inoculum, 250 ml. of the nematode inoculum, or 250 ml. of each of the two inocula. The pots were then filled with soil. For the gladiolus experiments three No. 1 size corms, variety Picardy or Dr. F. E. Bennett, were planted per pot. For the squash experiments, 3 seeds of the variety Farr's Benning White Bush were planted per pot, and for the tomato experiments, 3 seeds of the Marglobe variety. The squash and the tomato plants were thinned to one per pot. The date of the appearance of the first symptoms of Fusarium infection in each plant was recorded, and this information analyzed statistically. All of the plants were harvested while the noninoculated ones were still green. At this time the gladiolus had finished flowering, and the squash and tomato had fruited heavily. The number of days to harvest after planting was 99, 93, and 129 days for gladiolus, squash, and tomato, respectively. The roots of the tomato and squash plants inoculated with nematodes alone were scored for galling and given a relative root-knot index.[4]

In a second experiment, additional populations of *Heterodera marioni* were used with *Fusarium* on squash since considerable variability in pathogenicity exists among races[5]. These populations were obtained as follows: population 5, from parsnip, grown near Falls Church, Virginia; population 7, from a Castella rubber tree propagated in a Florida nursery; population 8, from tomato plants grown in a greenhouse at Beltsville, Maryland; and population 15, from gladiolus. The soil was infested, the plants grown, and the data recorded in the same manner as in experiment 1. There were but 16 pots of squash per treatment in the second experiment, and these plants were all dug 110 days after planting.

The root-knot indices, numbers of plants infected with *Fusarium*, and the average numbers of days prior to harvest when symptoms appeared, are recorded in table 1, for both experiments. Severe galling occurred in the tomato and squash as is indicated by the high root-knot indices obtained from the plants inoculated with nematodes only. The average number of days prior to harvest when symptoms appeared seems to be a reliable criterion of disease incidence. Thus, higher numbers indicate an earlier, more rapid incidence of infection; and consequently if *Heterodera marioni* speeds up the incidence of Fusarium infection or if it causes it to be more severe, the figures for the *H. marioni-Fusarium* combinations should be higher than for *Fusarium* alone. The data indicate this except in the case of squash in experiment 2. However, with but two exceptions, none of the differences are statistically significant. Incidence of Fusarium infection was significantly greater at the 5 per cent level in gladiolus when

[4] Taylor, A. L. Field methods of testing for root-knot infestation. Phytopath. 37: 85–93. 1947.

[5] Christie, Jesse R., and Florence E. Albin. Host-parasite relationships of the root-knot nematode, *Heterodera marioni*. I. The question of races. Proc. Helminth. Soc. Washington 11: 31–37. 1944.

combined with population 15 of *H. marioni* (treatment 4) and when combined with a mixture of populations 3B and 15 (treatment 12). These differences were not great, however, and it can be concluded that, under the conditions of these experiments at least, *H. marioni* had very little effect on the incidence of Fusarium infection. No conclusions can be drawn regarding the effect of different nematode populations.

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A WILT-INDUCING POLYSACCHARIDE FROM FUSARIUM SOLANI F. EUMARTII[1]

C. A. THOMAS

Among the several theories advanced in the last half-century to explain the wilting of plants affected with certain bacterial or fungus diseases, the toxin theory has been most generally accepted. A considerable amount of recent work has been concerned with culture filtrates of wilt organisms. It has been shown that these organisms, when grown upon synthetic media, produce toxic substances capable of wilting excised shoots of healthy plants. Although relatively few chemical analyses of culture filtrates have resulted in positive identification of the toxic substances, a number of different chemical compounds have been described as wilt-inducing substances.

The literature concerned with the wilt problem has been reviewed by Brown (1), Harris (7), and Gottlieb (6). Recent investigations have been made by Clauson-Kaas, Plattner, and Gäumann (2), Hodgson, Riker, and Peterson (9, 10, 11), Dimond (3), and Wolf and Wolf (13).

The purpose of this paper is to describe the properties and nature of a wilt-inducing substance present in culture filtrates of *Fusarium solani* f. *eumartii* (Carp.) Snyder and Hansen. This organism is the cause of a wilt and stem-end rot disease of potato.

EXPERIMENTAL WORK

Preparation of culture filtrates. A single spore culture of *Fusarium solani* f. *eumartii* was used in all experiments. The organism was grown on a modified Richard's solution of the following composition: KNO_3, 10 gm.; KH_2PO_4, 5 gm.; $MgSO_4$, 2.5 gm.; $FeCl_3$, 0.02 gm.; dextrose, 50 gm.; Haas and Reed's "A–Z" solution, 1 ml.; and distilled water, 1500 ml. The reaction of the nutrient solution was adjusted to pH 6.0 with K_2HPO_4 before sterilizing in an autoclave at 15 lb. pressure for 20 min. Roux flasks containing 200 ml. of the nutrient solution were inoculated with 3 loopfuls of conidia of the organism from cultures 10 days old. After incubation at room temperature for 14 to 20 days, the amber to purple culture solution, which was covered by a thick mycelial mat, was filtered through 3 layers of cheesecloth and then centrifuged at 5000 r.p.m. for 15 min. to remove most of the mycelial fragments and conidia. The pH of the culture filtrates was 8.0 to 8.5.

Wilting tests. Stems of healthy, rapidly growing Bonny Best tomato plants with 4 or 5 well-developed leaves were cut under water and then placed in test tubes containing 20 ml. of the solution to be tested.

The first experiments were run in the laboratory and in the greenhouse. Rather erratic results were obtained in these experiments and it was thought that such factors as light, temperature, humidity, and air movement were not constant enough to give consistent results. Several wilt tests were then performed in a cold storage room where temperature was fairly constant at 13° C. in the dark. The plants were illuminated by four 500-watt General Electric CX (infrared, mild ultraviolet) lamps. The plants were placed on a table 3 ft. below the lights and were separated from the lamps by a glass plate. A 10-in. fan was used to create air movement in the chamber. The fan was directed toward the side of the chamber and the deflected air passed over the plants. At the beginning of each wilt test the lights were turned on and the fan was started. In a period of 10 hr., the duration of a wilt test, the temperature in the chamber gradually rose from 13° C. to 32° C. and the relative humidity decreased. The lights and fan were turned off and the temperature was allowed to return to 13° C. before another test was run. Consistent results were obtained from tests performed in the cold-storage room under the described conditions.

A minimum of 3 cuttings was used to test individual solutions in most experiments. Unless otherwise stated each experiment was repeated at least 5 times. In each of the wilt tests checks consisted of plants in sterile nutrient solution. In no case did the checks wilt.

The results of several preliminary tests with unadulterated culture filtrates indicated that a wilt-inducing substance was present. Usually within 1 hr. the cuttings were partially wilted. There was some loss of turgidity in the petioles and the upper part of the stems causing the leaves and top part of the cuttings to droop. By the time the tests were concluded the stems were flaccid and the cuttings had collapsed. At this state a cutting was considered to be completely wilted although the leaflet blades were still somewhat turgid.

The following study of the properties of the wilt-inducing substance was made primarily to determine the type of substance involved.

Dialysis. One hundred ml. of culture filtrate were placed in a cellophane dialyzing tubing (Will Corp. No. 6598). A small marble was placed within the membrane for the purpose of stirring the solution. Dialysis was carried out in a Kunitz-Simms (12) rocking-table dialyzer. Distilled water was passed through the glass tube surrounding the membrane at the rate of 1 gal. per hour. The tubing was removed from the dialyzer after 24 hr. and the solution was tested for wilt-inducing activity. The pH of the culture filtrates was 8.0 to 8.5 before dialysis and 6.5 to 7.0 after dialysis. A total of forty-seven 100-ml. samples were tested after dialysis. In these tests a total of 184 tomato cuttings was used. Checks in each test included nondialyzed culture filtrate, sterile nutrient solution, and distilled water. Tomato cuttings in the dialyzed culture filtrate showed partial to complete wilt in 2 to 10 hr. Plants in the non-

dialyzed culture filtrate wilted somewhat more quickly and more severely. Later experiments showed that when mineral salts were added to the dialyzed culture filtrate the wilt-inducing activity was increased. No wilting occurred in shoots placed in the sterile nutrient solution or distilled water. No tests were run on the dialysate. Nondialyzed culture filtrates gave a strong test for reducing sugars with Fehling's solution but dialyzed filtrates gave a negative test, indicating that dialysis was effective in removing substances of small molecular weight. These results showed that the toxic substance had a relatively large molecular weight.

Filtration. Since filtration through cheesecloth and centrifugation does not completely remove all conidia and mycelial fragments it was thought advisable to determine whether or not the culture filtrate could be filtered through a bacterial filter without removing the wilt-inducing substance. Dialyzed and nondialyzed culture filtrates were filtered through Seitz, Berkefeld, and fritted-glass bacterial filters. Whatman No. 2 filter paper also was included in the tests. Tomato plants showed little or no wilt in filtrates passed through any of the bacterial filters. The toxic substance did pass through the filter paper. Culture filtrates were tested for filterability at pH 2.5 and 9.0. The pH was adjusted with either 0.1 N hydrochloric acid or sodium hydroxide. Filtrates were readjusted to pH 7.0 and tested for wilt-inducing activity. The toxic substance did not pass through the bacterial filters at either pH 2.5 or 9.0. Adjusting the pH of unfiltered solutions to either 2.5 or 9.0 did not inactivate the toxic substance. It is assumed that the molecules of the substance are either too large to pass such filters or that they are adsorbed on the filter.

Precipitation with ethyl alcohol. Nondialyzed culture filtrates were treated with 2 volumes of 95 per cent ethyl alcohol. The precipitate that formed was dried over calcium chloride and then redissolved in distilled water. Wilt tests showed that the toxic substance had been precipitated by the alcohol. Dialyzed culture filtrates were treated with 2 and 5 volumes of 95 per cent ethyl alcohol. The solutions turned slightly turbid but no precipitate resulted that could be collected by filtration or centrifugation. An attempt was then made to determine whether or not the toxic substance could be precipitated from the dialyzed culture filtrate by alcohol if the solution were more concentrated. Tubing containing a dialyzed culture filtrate was removed from the dialyzer and placed in front of an electric fan and the solution was evaporated to dryness. The residue from dialyzed filtrates was active and could be kept in the dried state for several weeks without any apparent loss in activity. The residue from 100 ml. of dialyzed culture filtrate was redissolved in 20 ml. of distilled water. Five ml. of this solution were run into 25 ml. of cold (0° C.) 95 per cent ethyl alcohol at the rate of one drop per second with vigorous stirring. A gummy, gray precipitate resulted which was removed by centrifugation and dried. The precipitate obtained from 100 ml. of dialyzed culture filtrate was redissolved in 100 ml. of distilled water and then tested

for wilt-inducing activity. The tests showed that the toxic substance was precipitated with the alcohol.

Solubility tests. The toxic substance appeared to be very soluble in water. Attempts were made to extract the toxic substance from dialyzed culture filtrates with organic solvents. Dried alcoholic precipitates from 50 ml. of dialyzed filtrate were shaken with 20 ml. of each of the following: ether, benzene, ethyl acetate, chloroform, and dioxan. The mixtures were allowed to stand for 6 hr. and then were filtered through paper. The filtrates were evaporated to dryness. An extremely small amount of residue was obtained from the filtrates. The residues collected on the filter papers and those obtained from the filtrates were redissolved in 25 ml. of distilled water and tested for wilt-inducing activity. In all cases the toxic substance appeared in the fraction not soluble in the organic solvent.

Effect of heat on nondialyzed culture filtrates. Since the wilt-inducing substance present in culture filtrates of *Fusarium solani* f. *eumartii* was reported by Goss (5) to be inactivated by boiling, experiments were performed to determine whether or not filtrates of the isolate used in these studies behaved similarly. One hundred ml. of nondialyzed culture filtrate (pII 8.0 – 8.5) were divided into two equal parts, one of which was placed in a steamer at 100° C. for 15 min. A precipitate formed on heating and this was removed by centrifugation. The heated and unheated solutions were adjusted to pII 7.0 with 0.1 N hydrochloric acid and the solutions were then tested for wilt-inducing activity. The results of such tests, involving 58 different cultures, showed that heating at 100° C. for 15 min. either partially or completely inactivated the wilt-inducing substance.

Effect of heat on dialyzed culture filtrates. The alcohol-insoluble precipitate from 100 ml. of dialyzed culture filtrate was dissolved in 80 ml. of distilled water. Heating this solution in the steamer for 15 min. to 2 hr. did not result in inactivation of the toxic substance. No precipitate formed on heating.

It was thought that the precipitate that resulted from heating non-dialyzed culture filtrates consisted largely of phosphates or other substances present in the nutrient solution that are insoluble in hot, alkaline solutions. To test this hypothesis, the alcohol-insoluble precipitate from 100 ml. of dialyzed culture filtrate was dissolved in 80 ml. of sterile, nutrient solution. This was divided into two equal parts, one of which was adjusted to pII 5.9 and the other to pII 7.5. Each of these two solutions was divided into two equal parts, one of which was heated at 100° C. for 15 min. in a steamer and the other left unheated. A precipitate formed in the solution heated at pH 7.5. This precipitate was removed by centrifugation. No precipitate formed on heating the solution at pH 5.9. Wilt tests showed that inactivation occurred only in the solution heated at pH 7.5. In subsequent experiments the precipitate that formed on heating at pH 7.5 was redissolved by adjusting the solution to pH 6.0 and allowing

it to stand at room temperature for 24 to 48 hr. Wilt tests showed that the toxic substance was present in this solution. It thus appeared that heating the toxic substance in an alkaline Richard's solution resulted in removal of the substance along with the precipitate or that the substance was insoluble in the presence of nutrients under these conditions. Heating in the absence of nutrients at pH 7.5 had no effect on the wilt-inducing activity.

Chemical nature of the wilt-inducing substance. Several qualitative chemical tests were run on the alcohol-insoluble precipitate obtained from dialyzed culture filtrates. The procedures followed in performing the various tests were those given by Hawk, Oser, and Summerson (8). The furfural tests were performed according to the procedure given by Fearon (4). A summary of the reactions is presented in table 1. A negative test

TABLE 1.—*Reactions exhibited by the alcohol insoluble precipitate from dialyzed culture filtrates of Fusarium solani f. eumartii*

Test	Reaction[a]	Test	Reaction[a]
Ninhydrin	−	Fehling's solution, before hydrolysis[b]	−
Biuret	−		
Millon's	−	Fehling's solution, after hydrolysis[b]	+
Xanthoproteic	¬?	Nitro-chromic, after hydrolysis[b]	+
Trichloroacetic acid	−	Furfural	
Phosphomolybdic acid	−	Thymol, 15% HCl	−
		Thymol, 35% HCl	+
Molisch	+	Orcinol, 15% HCl	−
		Orcinol, 35% HCl	+
Osazone after hydrolysis[b]	+	Iodine	−
Precipitation with C₂H₅OH	+		

[a] + positive reaction;—negative reaction; ¬? weak or incomplete reaction.
[b] 0.5 N HCl at 100° C. for 2 hr.
[c] Glucosazone type crystal formed.

for protein was obtained in the ninhydrin, biuret, and Millon's tests. The xanthoproteic reaction was weak and could not be interpreted as definitely positive or negative. No precipitation occurred with trichloroacetic acid or phosphomolybdic acid. Iodine gave no color reaction. The Molisch test for carbohydrate was positive. Aqueous solutions of the precipitate gave a negative test for reducing sugar with Fehling's solution. Hydrolysis was carried out by dissolving the alcohol-insoluble precipitate from 100 ml. of dialyzed filtrate in 0.5 N hydrochloric acid and heating for 2 hr. at 100° C. Solutions of hydrolyzed material gave a strong test for reducing sugar with Fehling's solution. The nitro-chromic reaction was positive after hydrolysis. In the furfural tests thymol gave no reaction in 15 min. at 100° C. with 15 per cent hydrochloric acid but gave a carmine color with concentrated hydrochloric acid at the same time and temperature. Orcinol reacted similarly. The furfural reactions indicated that the sugar

was an aldohexose. Glucosazone crystals were formed in the phenylhydrazine reaction with hydrolyzed material. The phenylhydrazine test was negative with unhydrolyzed material.

Tests were performed to determine whether or not hydrolysis of the alcohol-insoluble precipitate inactivated the wilt-inducing substance. The alcohol-insoluble precipitate from 100 ml. of dialyzed culture filtrate was dissolved in 10 ml. of distilled water. This solution was divided into two equal parts. Sufficient concentrated hydrochloric acid was added to one part so that the concentration was 0.5 N. Both solutions were heated at 100° C. for 2 hr., then cooled, and adjusted to pH 7.0. Thirty ml. of distilled water were added to each of the two solutions which then were tested for wilt-inducing activity. Complete inactivation of the wilt-inducing substance resulted from the acid hydrolysis. The solution heated in the absence of acid was fully active. The acid-hydrolyzed solution gave a strong test for reducing sugar with Fehling's solution and the test with the unhydrolyzed solution was negative.

Results of the preceding tests indicated that the wilt-inducing substance was possibly a polysaccharide of high molecular weight or a mixture of polysaccharides. A polysaccharide was always associated with fractions exhibiting wilt-inducing activity. The polysaccharide was partially precipitated with saturated ammonium sulphate. In all cases the most active fraction contained the most polysaccharide.

Amount of material present in culture filtrates. The amount of material obtained by alcoholic precipitation from dialyzed filtrates was determined. The precipitates obtained from three 100-ml. portions of dialyzed filtrates were dried in an oven at 90° C. for 24 hr., then cooled in a desiccator at room temperature, and weighed. The weights of the precipitates were 46.2, 45.8, and 44.1 milligrams.

Nature of action of the wilt-inducing substance. Tomato plants wilted in culture filtrates did not recover when placed in distilled water. Sections measuring ¼ in. or more were cut under water from the basal part of stems of wilted cuttings and the plants were placed in distilled water. The plants completely recovered from their wilted condition within 2 to 10 hr. Plants wilted in dialyzed or nondialyzed filtrates or in aqueous solutions of the alcohol-insoluble precipitate from dialyzed filtrates behaved alike. The wilt produced by these solutions affected primarily the stems of the cuttings. These results suggested that the substance possibly causes wilt by plugging the vessels of the stem.

DISCUSSION

The evidence presented indicates that at least one of the wilt-inducing substances present in the culture filtrates is a polysaccharide. The substance is either nondialyzable or very slowly dialyzable. This fact simplified the partial purification since substances of smaller molecular size were easily removed. It is possible that there are other dialyzable, wilt-inducing

substances present in such filtrates. Although no attempt was made to compare directly the wilt-inducing activity of filtrates before and after dialysis, the results indicated that the nondialyzable substance accounted for a large part of the activity of the culture filtrates. Dialyzed and nondialyzed filtrates produced a similar type of wilt.

The thermal inactivation of nondialyzed, alkaline filtrates was interpreted incorrectly at first as indicating that the toxic substance was an enzyme or protein. It was shown that the polysaccharide obtained from dialyzed filtrates by alcoholic precipitation also was inactivated by heat when dissolved in an alkaline Richard's solution. The inactivation appeared to be due to removal of the polysaccharide from solution along with phosphates and other substances precipitated by heating the alkaline nutrient solution. The inactivation was not thought to be due to lowering the osmotic concentration by precipitation of the phosphates, since aqueous solutions of the polysaccharide were active.

Hodgson, Peterson, and Riker (9, 10) reported wilt-inducing polysaccharides from a number of organisms not associated with wilt diseases. They found the type of wilt produced was correlated with molecular size and solubility. Results of other experiments indicated that the wilting mechanism may be mostly physical (11). In the present study the toxic substance was found to cause wilting primarily of the stems and petioles of tomato cuttings. Since wilted cuttings recovered when placed in distilled water if sections ½ in. or more in length were cut from the basal part of the stem, it appeared that the wilting was possibly caused by a physical blocking in the base of the cut stem.

Whether or not wilt-inducing substances present in culture filtrates of wilt organisms are involved in the production of wilt in naturally infected plants is not known. With a knowledge of the substances which may be involved, further investigations with naturally infected plants may lead to more definite conclusions.

SUMMARY

A wilt-inducing substance produced in liquid cultures of *Fusarium solani* f. *eumartii* was found to be nondialyzable, not filterable through bacterial filters, soluble in water, and relatively insoluble in several organic solvents.

The substance was precipitated from nondialyzed culture filtrates by the addition of two volumes of ethyl alcohol. Precipitation with alcohol from dialyzed filtrates was effected only after concentration of the filtrates.

The wilt-inducing substance was thermostable in neutral, aqueous solution and labile in strong acid solution. It was partially to completely inactivated by heat in nondialyzed, alkaline filtrates and in alkaline Richard's solution.

The toxic substance was found to induce wilting primarily in the stems and petioles of tomato cuttings. The leaflets generally were less affected.

Wilting appeared to be caused by a condition brought about in the basal part of the cut stem, possibly a physical blocking. Wilted cuttings did not recover when placed in distilled water unless sections ½ in. or more in length were cut from the basal part of the stem.

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CONTROL OF BLACK ROT IN WASHED, UNCURED
SWEET POTATOES

W. J. Martin, J. M. Lutz, and G. B. Ramsey[1]

Results of laboratory tests for the control of black rot caused by *Cerastomella fimbriata* (Ell. and Hals.) Elliott in washed, uncured sweet potatoes[2,3] indicated that a momentary dip of the potatoes in a 1 per cent solution of borax would give adequate control of this disease. To further test the applicability of the borax-dip treatment, tests were made under commercial conditions. The purpose of this paper is to report the results of shipping and holding tests made in 1948 with borax-treated sweet potatoes and with sweet potatoes treated with an emulsion wax containing a fungicide, 2 per cent Dowicide A (sodium orthophenylphenate). The latter treatment was being used by several shippers who believed it was effective in controlling black rot.

Individual growers' lots of sweet potatoes containing black-rot lesions were selected for the tests. In preparing the test crates, the individual roots with visible lesions were graded out and were not included in the packed crates. Two 50-lb. crates, one treated and one untreated, were packed from each grower's lot. A total of 13 lots was used in the borax tests and 10 additional lots were used in the wax-fungicide tests. The experimental crates were packed at commercial packing sheds in Southwest Louisiana.

The tests with the borax treatment were made at a packing shed equipped with a dip tank. In this installation the sweet potatoes were washed by means of an overhead spray system and carried on chain rollers into the dip tank containing a solution of approximately 1 per cent borax. Another system of chain rollers carried them out of the tank to a conveyor belt, from which they were packed into the paper-lined commercial crates for shipment.

Tests with the emulsion wax containing a fungicide were made at two packing sheds equipped with foam waxers. The wax was applied to the sweet potatoes immediately after washing and just before packing, at the rate of 3 to 5 gal. per carload (504 bu.).

The experimental crates from six lots in the borax test and six lots in the wax-fungicide test were shipped to Chicago in commercial truck- or car-lots. Notes on the percentage of black rot in the treated and untreated

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TABLE 1.—*Percentages of sweet potatoes that developed black rot in treated and untreated crates from 12 lots of sweet potatoes shipped to Chicago in August and September, 1948*

| Lot number[a] and treatment | Percentages of sweet potatoes with black-rot lesions | | | |
| | On arrival | | 1 wk. after arrival[b] | |
	Treated	Untreated	Treated	Untreated
Borax treatment				
1	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0
3	0.0	0.7	0.0	0.7
4	0.0	0.0	1.0	0.0
5	0.5	0.0	2.1	4.0
6	3.7	0.6	3.7	5.0
Wax-fungicide treatment				
7	1.4	2.4	20.5	34.6
8	0.8	1.3	39.8	14.2
9	0.0	2.2	27.7	42.0
10	0.0	0.0	0.0	0.0
11	0.0	0.0	0.0	0.0
12	0.0	0.0	0.0	0.0

[a] Designating different crops.
[b] Includes lesions that developed in transit.

crates were recorded, on arrival (about 3 days after packing), and 1 week after arrival (Table 1). While relatively little black rot developed in either the treated or untreated crates of the borax test, considerable black rot developed, particularly after arrival, in both the treated and untreated crates from three lots in the wax-fungicide test. It can be noted that borax-treated lot number 6 had 3.7 per cent black rot on arrival but there was no increase

TABLE 2.—*Percentages of sweet potatoes that developed black rot in treated and untreated crates from 11 lots of washed, uncured sweet potatoes stored for 10 days in Southwest Louisiana in 1948*

| Lot number[a] and treatment | Percentage with black rot | |
	Treated	Untreated
Borax treatment		
1	0.0	12.9
2	0.0	1.2
3	0.0	11.9
4	0.0	14.8
5	0.0	0.0
6	0.0	0.0
7	0.0	10.7
Averages	0.0	8.0
Wax-fungicide treatment		
8	47.9	5.9
9	91.1	91.2
10	80.7	87.2
11	82.4	90.7
Averages	76.1	68.3

[a] Designating different crops.

during a 1-wk. holding period after arrival. It is probable that a slight infection at time of shipping would account for this.

Paired experimental crates from seven other lots that were treated with borax and from four lots receiving the wax-fungicide treatment were held in storage locally. Each lot was from a different crop. Notes on the percentages of black rot in these were recorded 10 days after packing (Table 2). Considerably more black rot developed in the lots held locally than in those that were shipped to Chicago. However, no black rot was found in any of the borax-treated crates, although an average of 8 per cent of the potatoes in the comparable untreated crates developed black rot lesions. Abundant black rot developed in the four lots in the wax-fungicide test, and the treated and untreated crates were equally affected.

No evidence of injury was noted in the borax-treated sweet potatoes. A darkening of bruised areas was noted in some cases on those given the wax-fungicide treatment.

The results of these tests indicate the effectiveness of the borax-dip treatment for the control of black rot in washed, uncured sweet potatoes, and the ineffectiveness of the emulsion wax containing sodium orthophenylphenate.

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SOIL TREATMENTS WITH CHLOROPICRIN, D–D, AND URAMON FOR CONTROL OF THE ROOT–KNOT NEMATODE[1]

C. N. Clayton and D. E. Ellis[2]

Several chemicals such as chloropicrin, ethylene dibromide, and a mixture of dichloropropane and dichloropropene are known to be effective in control of *Heterodera marioni* (Cornu) Goodey (1, 4, 5, 6). Nevertheless, many questions regarding the use of such materials remain unanswered (1, 3, 5). One of these questions deals with the time of application. It is known that a certain period of time must elapse following the application of many soil treatment materials before crops can be planted without risk of injury (1, 5). However, it is not always feasible or practical to apply a treatment at a certain season of the year. Experiments herein reported were conducted to determine the effect of applying certain chemical nematocides to soil in spring, summer, and fall of one year upon the control of root-knot and upon the growth and yield of snap bean and tomato planted 14, 10, and 7 months after treatment and of cantaloupes planted 26, 22, and 19 months after treatment.

MATERIALS AND METHODS

The nematocides[3] used and the rates of application in pounds per acre were 1) Uramon, 2420; 2) Uramon, 4840; 3) D-D, 200; 4) D-D, 400; 5) D-D, 600; and 6) chloropicrin, 400. Nontreated plots were included as controls. Three replicates of each of the six treatments and the nontreated control were used for each of the three dates of treatment, March 11, July 12, and October 12, 1946, at each of 2 locations. The plot size was 6 by 12 ft. with 1-ft. alleys between plots. The soil type at location 1 (McCullers, N. C.) was Ruston sandy loam and at location 2 (Eagle Springs, N. C.) Norfolk fine sand. The soil at both locations was infertile and low in organic matter. The root-knot nematode population at McCullers was very high and uniform in distribution, whereas at Eagle Springs it was only moderately high and irregularly distributed.

The Uramon was broadcast uniformly by hand just prior to spading the soil about 6 in. deep. Using a hand applicator the D-D and chloropicrin were injected at 12-in. intervals and 5 in. deep into the soil. Im-

mediately after injection of chloropicrin the surface of the soil was sprin-
kled with water in order to seal the fumigant in the soil.

Following treatment, weeds were permitted to grow on the plots until
they were prepared for planting in the spring of 1947. On the chloro-
picrin-treated plots crab grass was the predominating species and it grew
very well. Although the population of weeds and grass on the Uramon-
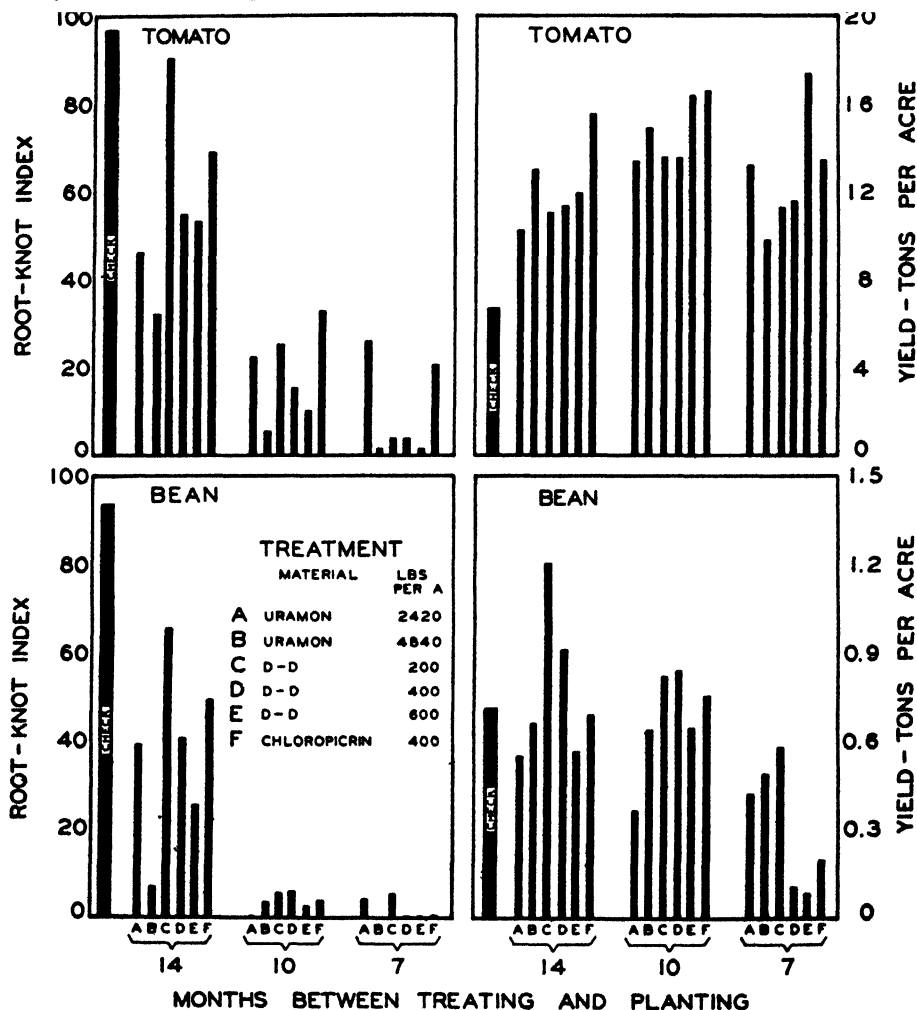
FIG. 1. Effect of soil treatments applied 14, 10, and 7 months prior to planting
upon control of root-knot and upon yields of tomato and snap bean.

treated plots was greatly reduced, the plants that grew were strongly
stimulated by the nitrogen from the Uramon. All plots were fertilized
uniformly with 5–10–5 fertilizer at the rate of 2000 lb. an acre prior to
planting.

Six Marglobe tomato plants grown in 2½-in. clay pots under glass and
100 Tendergreen snap bean seeds were planted on each plot in May, 1947.

Stand counts of beans were taken and ratings on the plant vigor of beans and tomatoes were made independently by three persons several times during the season. The yields of marketable green beans and tomatoes were taken. After harvest the roots of each plant were examined for root-knot. The bean plants were examined on July 29 and the tomatoes on August 18. The relative severity of root-knot was rated and indexes (2) for the different plots were calculated. The data from the 2 locations were generally in agreement. However, only the data for the Mc-Cullers test are presented here because at that location the infestation of the root-knot nematode was distributed uniformly. The data were analyzed by the Department of Experimental Statistics, North Carolina State College. Only statistically significant data are stressed.

RESULTS

Effect on root-knot control. The data on root-knot control (Fig. 1) show that all treatments applied in October (7 months prior to planting) reduced root-knot to a very low level in comparison with the nontreated. For most treatments applied in July (10 months before planting) the average root-knot indexes, particularly for tomato, were somewhat higher than for the respective treatments applied in October. The indexes for the treatments made in March (14 months prior to planting) were much higher than those for treatments applied in July or October. This higher incidence of root-knot on the plots treated in March was most likely the result of a large increase in the population of the root-knot nematode during the summer months following treatment. The data in figure 1 indicate that the higher rates of D-D and of Uramon were generally more effective than the lower rate.

Effect on yields. All materials at all dates of application reduced the nematode infestation sufficiently to permit much better growth and yield of tomatoes than on nontreated plots. Although the degree of root-knot control on beans was similar to and somewhat better than that on tomatoes, this was not generally reflected in increased yields. In fact, bean yields from all plots treated 7 months before planting were lower than those from the nontreated, indicating that some deleterious effect on growth of beans resulted from some of the treatments. This was somewhat more noticeable on plots treated with chloropicrin and the higher rates of D-D seven months before planting than on those treated 10 or 14 months prior to planting (Fig. 1). This deleterious effect and the control of root-knot were converse factors affecting the growth of beans. This explains why the highest yields occurred on plots which were treated with D-D at the lowest rate and yet had the highest root-knot index. The yield data were closely paralleled and substantiated by those on plant vigor. Therefore the latter are not given.

Effect of water-seal with D-D. Keeping the soil surface wet for a few days after chloropicrin has been injected has been found (4) to increase

the effectiveness of the fumigant by sealing it in the soil until the nematodes are killed. In conjunction with the preceding experiment, D-D at

TABLE 1.—*Comparative effectiveness of D-D applied at 200 lb. and 400 lb. per acre with or without a water seal 14, 10, and 7 months prior to planting, upon root-knot control and upon yield of tomato and snap bean*

Treatment			Time of application in 1946	Root knot index		Yield per acre	
Material	Rate of application per acre	Water seal		Tomato	Bean	Tomato	Bean
	Lb.					*Tons*	*Tons*
D D	200	No	March	91	66	11 0	1 20
			July	25	5	13 6	0 82
			October	4	6	11 3	0 57
D D	200	Yes	March	79	36	16 7	1.24
			July	16	22	14 8	0 75
			October	4	2	13 2	0.47
D D	400	No	March	55	41	11.3	0 91
			July	11	6	13 6	0.85
			October	4	1	11 6	0.10
D-D	400	Yes	March	37	15	15 0	1 06
			July	16	6	14 1	0.52
			October	0	1	11 9	0.13
None (check)				97	94	6 5	0 71
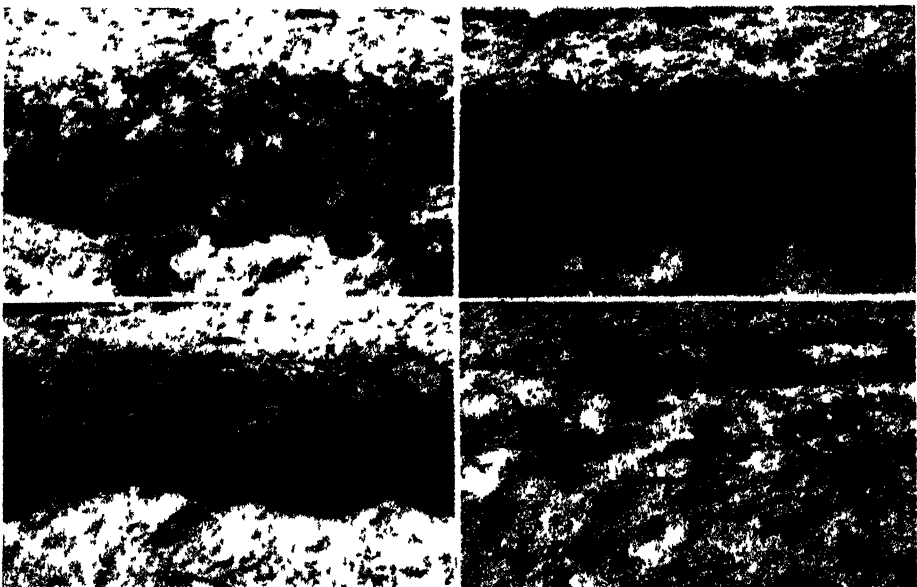
FIG. 2. Cantaloupe plants in 1948 on plots treated on October 12, 1946. Upper left, chloropicrin, 400 lb. per acre, plus a water seal. Upper right, D-D, 400 lb. per acre. Lower left, Uramon, 2420 lb. per acre. Lower right, no treatment (plants killed by root-knot).

200 and 400 lb. per acre with and without a water-seal was applied 14, 10, and 7 months prior to planting of tomato and snap bean. The results (Table 1) of these tests at McCullers indicate that root-knot was well controlled with both dosages of D-D applied 7 or 10 months prior to planting and with or without a water-seal. The water-seal improved the control only when used in the March treatments, 14 months prior to planting.

The bean yields appeared not to be significantly affected by water-seal. The plots treated with D-D at 200 lb. outyielded those treated at the 400-

TABLE 2.—*Effect of soil treatments applied on March 11, July 12, and October 12, 1946 upon root-knot control and upon yield of cantaloupes in 1948*

| Material | Treatment | | Time of application in 1946 | Root knot index | Yield of cantaloupes per plot (number) |
	Rate of application per acre (in lbs.)	Water-seal			
Uramon	2420	No	March	81	4
			July	41	17
			October	29	13
Uramon	4840	No	March	85	9
			July	19	17
			October	46	13
D-D	200	No	March	100	5
			July	69	12
			October	70	16
D-D	200	Yes	March	100	6
			July	88	17
			October	34	33
D-D	400	No	March	95	6
			July	86	20
			October	44	23
D-D	400	Yes	March	81	17
			July	74	19
			October	15	26
D-D	600	No	March	91	4
			July	63	23
			October	8	18
Chloropicrin	400	Yes	March	95	13
			July	87	14
			October	71	12
None (check)				100	3

lb. rate. The effect of the latter in the October application was to reduce drastically the yield to about 1/8 that of the March treatment and about 1/7 that of the nontreated. These low yields are not attributable to root-knot. There was a highly significant difference in yields of beans for the three dates of treatment.

Tomato yields from plots treated 7 or 10 months prior to planting were not affected by water-seal, while, on those treated 14 months ahead of planting, a water-seal resulted in a significant increase in yield. The yield of marketable tomatoes on nontreated plots in 1947 was approxi-

mately ½ that on plots treated with D-D at 200- or 400-lb. per acre with or without a water-seal.

Effect upon root-knot and yield of cantaloupes planted second year after treatment. Cantaloupes were planted in 1948 on the plots treated in March, July, and October of 1946 and cropped to tomatoes and beans in 1947. Because of less injury from root-knot even in the second year after treatment, the stand and growth of plants on treated plots were much better than on nontreated plots (Fig. 2). Furthermore, the data on control of root-knot and on yield (Table 2) show that the effect of treatment persisted into the second year, although it was less pronounced than in the first year after treatment. In general, the treatments which were the more effective against root-knot in 1947 continued to be the more effective ones in 1948.

All treatments resulted in increased yields of cantaloupes in comparison with the nontreated. The yields from all July or October treatments were 4 to 10 times greater than from the check. However, the increases from the treatments applied in March 1946 were relatively small except with chloropicrin or with D-D at 400 lb. plus a water-seal.

SUMMARY

1. Applications of chloropicrin at 400 lb.; D-D at 200, 400, and 600 lb.; and Uramon at 2420 and 4840 lb. per acre were made to sandy loam soil in March, July, and October of 1946 prior to the planting of tomatoes and snap beans in 1947 and cantaloupes in 1948.

2. In comparison with the nontreated checks, all October and July treatments and some of those applied in March effectively reduced root-knot. In general, the treatments applied in July or in October resulted in better control than those applied in March.

3. In the March application, Uramon at 4840 lb. per acre was more effective in controlling root-knot than at 2420 lb., while D-D at 600 or 400 lb. per acre gave better root-knot control than at the 200-lb. rate.

4. In comparison with the nontreated check, all treatments resulted in much better growth and higher yields of tomatoes. Similar results were not obtained with beans although root-knot control with the two crops was similar.

5. Some toxic or deleterious effect on growth and yield of beans resulted from treatment with D-D, chloropicrin, or Uramon.

6. Root-knot was well controlled with D-D applied 7 or 10 months prior to planting at rates of 200 and 400 lb. per acre with or without a water-seal.

7. The beneficial effect of treatment persisted into the second year and was reflected in improved growth and yield.

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     RALEIGH, NORTH CAROLINA

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EFFECTS OF SOIL TREATMENTS WITH URAMON AND CERTAIN FUMIGANTS UPON PLANT GROWTH AND INCIDENCE OF ROOT–KNOT[1]

D. E. Ellis, C. N. Clayton, and R. G. Owens[2]

Root-knot, caused by *Heterodera marioni* (Cornu) Goodey, is one of the most serious diseases affecting southern vegetable crops. In North Carolina it is a particularly important problem in home gardens and frequently causes heavy damage in commercial plantings. The disease is also an important factor in the establishment of new peach orchards. While control of root-knot by crop rotation is fairly effective (4), the method is often not applicable to the above crops. Many chemical soil treatments have shown promise against root-knot (1,.2, 8, 10), but many questions regarding their use are as yet unanswered (7, 12). Field studies were made to obtain more information on the effects of rate and time of application of various materials upon root-knot control and the response of various plants, on possible cumulative toxicity to plants resulting from repeated applications in successive years, and on methods of reducing injury to crops following treatment with Uramon.

MATERIALS AND METHODS

For the tests outlined below the nematocidal materials were supplied by the manufacturers.[3] Uramon, Cyanamid (calcium cyanamide), peanut-hull meal, cottonseed meal, and manure were broadcast evenly by hand and immediately disked or spaded into the soil to a depth of about 6 in. Rye was sown broadcast by hand, covered with a disk or hand rake, and the resulting crop disked or spaded into the soil about a month in advance of planting. The volatile fumigants were injected into the soil at 12-in. intervals and 5 in. deep with a hand injector. A water seal was used on all chloropicrin plots. All materials were tested in the experiments at McCullers, North Carolina. One experiment was duplicated at Eagle Springs, North Carolina. In all tests care was taken to prevent transfer of soil from one plot to another by providing adequate borders and drainage furrows between plots, and by using care in cultivation, most of which was done by hand. All plots received equal applications of commercial

5–10–5 or 6–8–6 fertilizer at rates of 1500 to 2000 lb. per acre applied in the row or broadcast by hand from 2 to 7 days prior to planting.

The plants used in the various tests included Tendergreen snap bean, Marglobe tomato, Clemson Spineless okra, and Lovell peach. The tomato plants were started in steam-disinfested soil under glass. Stand counts, growth measurements, and yields of the three vegetable crops were obtained. In certain tests a further measure of vegetative growth was obtained by weighing the green plants after harvest was complete. At the end of the harvest period the plants were dug, the roots rated for root-knot, and root-knot indexes (6) calculated. The data were analyzed by the Department of Experimental Statistics, North Carolina State College.

SOIL TREATMENTS WITH URAMON

Although soil treatment with Uramon is known to be highly effective in control of root-knot (3, 5, 7, 11), its use often results in stunted plants

TABLE 1 —*Effect of soil treatment with Uramon and certain supplementary materials upon control of root knot and upon yield of tomatoes*

Treatment[a]	Root knot index		Yield of tomatoes (tons per acre)
Materials and rates (lb. per sq. yd.)	1946	1947	1946
Uramon 1	8.7	28.7	5.2
Do + Cyanamid ½	11.8	36.6	4 0
Do + Peanut-hull meal 1	10.6	52.5	7.7
Do + Cottonseed meal 1	13.7	35.4	9.1
Nontreated (check)	98.5	100.0	3.1
L.S.D. (0.05)	18.9	25.5	2.1

[a] The soil was Norfolk sandy loam heavily infested with root-knot nematodes. Four replicate plots, 0.004 acre each, were used for each treatment. The Uramon and Cyanamid were applied October 29, 1945, and the other materials on February 25, 1946. Tomato plants were set on the plots on May 8, 1946, and May 5, 1947.

and low yields in the first crop after treatment (3, 7, 11). The addition of certain organic materials to counteract such injury has shown promise (7, 9). Results of a preliminary test in 1945 indicated that the application of finely ground peanut hulls or cottonseed meal to Uramon-treated soil was somewhat effective against the deleterious effects of this treatment on the first crop after treatment (7).

Table 1 shows the results of an experiment in which Uramon plus Cyanamid, Uramon plus peanut-hull meal, and Uramon plus cottonseed meal were applied in the fall and winter of 1945–46 prior to planting crops in the spring of 1946 and 1947. Although all treatments effectively reduced the incidence of root-knot in 1946 in comparison with the check, there were no significant differences between the root-knot indexes for the treated plots. This was still the case in 1947, although on the treated plots there was considerable increase in root-knot in comparison with the

respective indexes for 1946. The average yield of tomatoes in 1946 from plots treated with Uramon plus peanut-hull meal and Uramon plus cottonseed meal were 2.5 to 3 times that from the nontreated plots.

The phytotoxic effect of the Uramon treatment was found to vary with different soil types in other experiments (3). The effectiveness and phytotoxicity of treatments with Uramon plus several supplements was further studied on Norfolk sandy loam soil very heavily infested with root-knot nematodes at McCullers, North Carolina, and on Norfolk fine sand practically free from root-knot nematodes at Eagle Springs, North Carolina. The treatments and subsequent cropping history were similar at both locations.

The results of the experiment at McCullers are shown in table 2.

TABLE 2.—*Effect of Uramon plus several supplementary treatments upon control of root-knot and yields of tomato, okra, and snap bean at McCullers, North Carolina*

Treatment[a]	Average root-knot index	.Av. yields per acre		
Materials and rates (lb. per sq. yd.)		Tomato (tons)	Okra (bu.)	Bean (bu.)
1. Uramon ¼	47	4.83	173	90
2. Do ½	55	4.59	202	90
3. Do ¼ + Rye cover crop	49	7.59	226	94
4. Do ½ + do	35	5.94	239	104
5. Do ¼ + Peanut-hull meal 1	56	5.34	202	67
6. Do ½ + do	37	5.31	208	69
7. Do ¼ + Manure 4	56	5.01	193	65
8. Untreated check	87	3.75	82	82
L.S.D. (0.05)	16	1.40	77	20

[a] Four replicate plots, 0.007 acre each, were used for each treatment. The Uramon was applied on October 1, 1947, and the other materials were applied and the rye sowed on October 30, 1947. Peach pits and 3 peach seedlings, 7 tomato plants, 100 bean and 200 okra seeds were planted on each plot in the spring of 1948. Low soil moisture from May to July reduced and retarded the development of root-knot and of snap bean.

The root-knot indexes for okra, tomato, and peach are averaged since they showed similar trends. While all treatments significantly reduced root-knot, in comparison with the nontreated check, none reduced it to a very low level. However, yields of tomato and particularly of okra showed beneficial effects from most treatments in comparison with the check. The highest yield of bean, of okra, and of tomato was obtained on Uramon plus rye plots. Despite the fact that Uramon at the lowest rate permitted root-knot incidence to be fairly high, the treatment reduced it sufficiently to allow good growth of okra and peach whereas the plants in the nontreated plots were stunted and dead (Fig. 1). This increase in growth is believed to be primarily the result of root-knot control. The plants on nontreated plots, where root-knot was severe, were stunted and killed prematurely but, in the experiment at Eagle Springs, where root-knot was definitely not a factor, the best growth occurred on the nontreated plots (Fig. 2).

FIG. 1. Okra and peach plants on plots treated with Uramon, ¼ lb. per sq. yd., on October 1, 1947, followed by a winter cover crop of rye (left), in comparison with those killed by root knot on nontreated plots (right). Photographed August 27, 1948. McCullers, North Carolina.

The results at Eagle Springs are summarized briefly. The root-knot indexes were practically zero for all treatments and the nontreated checks with all crops. The growth and yields of the beans were similar for the various treatments. The yields of tomatoes appeared to be affected very
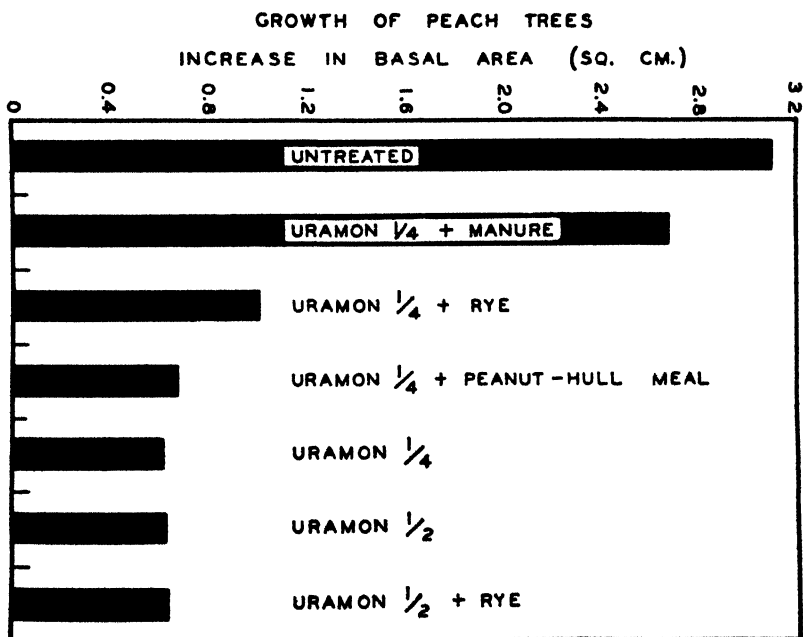
FIG. 2. Growth of peach trees in first season following soil applications of Uramon at ¼ and ½ lb. per sq. yd. on Oct. 1, 1947, and of addition of supplements to Uramon-treated plots on November 1, 1947. Eagle Springs, North Carolina.

little by any of the treatments. The phytotoxic effect of the Uramon treatments was severe upon peach trees set on December 23, 1947. At this location the addition of manure was the only amendment that counteracted this effect on peach trees (Fig. 2).

SOIL TREATMENTS WITH VOLATILE FUMIGANTS

Rapid strides have been made in the development and use of volatile chemicals to disinfest soil prior to the planting of root-knot-susceptible crops (2, 10). However, many questions regarding the use of such materials remain inadequately answered (7, 12). Although fumigants applied at commercially practicable rates will reduce root-knot, they will neither eradicate nematodes from the soil nor prevent their re-entry (2, 10).

TABLE 3.—*Effect of soil treatment with volatile fumigants applied in 3 successive years upon root-knot control and yields of tomato and snap beans*

Treatment[a]	Average root-knot index			Average yield (bushels per acre)			
				Tomato		Snap bean	
	1946	1947	1948	1947	1948	1947	1948
Chloropicrin	0.2	1.0	0.1	446	197	22	24
D-D	1.5	9.9	9.1	540	203	41	61
Ethylene chlorobromide	0.1	2.5	0.2	541	203	140	68
Ethylene dibromide (Dowfume W-10)	3.3	11.3	2.8	511	210	103	98
Ethylene dibromide (Iscobrome D)	10.0	4.6	1.4	422	313	87	67
Untreated (check)	71.3	95.5	70.0	254	110	90	71
L.S.D. (0.05)	9.6	9.2	8.5	147	91	45	N.S.

[a] All materials were applied at a rate of 2.6 ml. per sq. ft. This rate expressed on an acre basis is approximately 400 lb. of chloropicrin and ethylene chlorobromide, 300 lb. of D-D, and 220 lb. of Dowfume W-10 and Iscobrome D. The dates of application were May 16–20, 1946; May 2, 1947; and April 21, 1948. The soil was Ruston sandy loam uniformly and moderately infested with root-knot nematodes. Two replicate plots, 0.003 acre each, were used for each treatment. Beans and tomatoes were planted about 1 month after treatment in each of the 3 years.

Therefore, for continuous control the treatment must be repeated. Little information is available on possible cumulative deleterious effects of successive treatments applied to the same soil. Furthermore, additional information is needed regarding the response of various crop plants to fumigants applied at different rates and seasons. While certain fumigants effectively control root-knot, this benefit is sometimes nullified by injury. Recently it has been shown (3) that while certain nematocides reduce root-knot to about the same level on bean and tomato, the response of the two crops is vastly different. Also, there were marked differences in control of root-knot and in plant growth and yield of crops following treatment in the spring, summer, or fall. The objectives of the following experiments were to throw more light upon these problems.

Treatment in successive years. Results from the use of certain volatile fumigants applied to the same plots in 3 successive years are given in table 3. The average root-knot indexes show that all treatments reduced root-knot to a low level. However, in all plots the disease was present, at
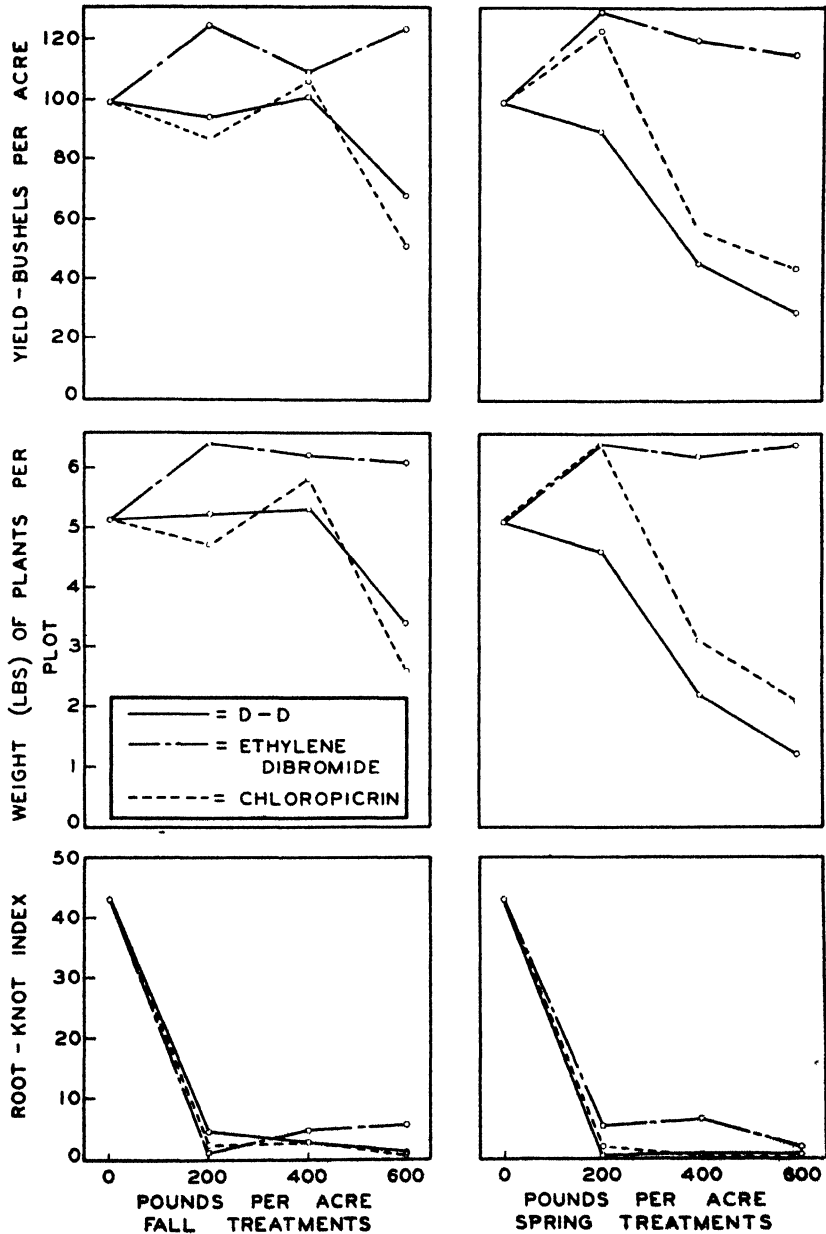
FIG. 3. Effect of soil treatment with certain fumigants applied at different rates in fall and in spring prior to planting of snap beans, upon root-knot, yield, and weight of green plants.

least in trace amounts, in the third year. After the first year all treatments markedly increased yields of tomatoes. Although yields fluctuated considerably from year to year, there was no clear-cut indication of cumulative phytotoxic effects. There was, however, an indication that the chloropicrin and D-D treatments depressed the growth and yield of beans.

Rate and time of application. Fall and spring applications of chloropicrin, D-D, and ethylene dibromide (Iscobrome D), each at rates of 200, 400, and 600 lb. per acre, were compared. The soil (Norfolk sandy loam) was irregularly and only lightly to moderately infested with root-knot nematodes. The plot size was 0.003-acre and each treatment was replicated 4 times in randomized blocks. The fall applications were made on October 3, 1947 (soil temperature 65° F.), and the spring applications were made on March 26, 1948 (soil temperature 63° F.). During the 5 months following the fall application the soil moisture was at abnormally high levels. On each plot 200 bean seeds (100 in each of 2 rows) were planted on May 6. The bean seeds for planting one row were inoculated with the proper nodule-forming bacteria for snap bean; those for the other row were not inoculated. Since no noticeable differences in stand, vigor, yield, or nodulation could later be detected, the data for inoculated and non-inoculated bean rows were combined. The bean plants were examined for root-knot in July.

The results presented in figure 3 show that while all three materials at each rate applied in spring or fall reduced root-knot to a very low level, some treatments markedly depressed growth and yield. The data on yield and on green weight of plants show that injury resulted from treatment with D-D and chloropicrin, applied at the highest rate in the fall and at the two higher rates in the spring. The injury was greatest at the highest rate. Treatments with ethylene dibromide appeared to be noninjurious.

Yields of beans on plots treated with D-D at 200 lb. per acre in the spring were significantly lower than on those treated with ethylene dibromide or chloropicrin at the same time and rate.

These data confirm previous work (3) showing that date of treatment, dosage, and response of certain crops are important factors to be considered in soil fumigation.

SUMMARY

1. In field experiments, root-knot was effectively reduced and yields of tomato were markedly increased by soil treatment with Uramon, chloropicrin, D-D, ethylene dibromide, or ethylene chlorobromide. Annual applications of the latter four materials in three successive years to the same plots neither eradicated the root-knot nematode nor resulted in any noticeable cumulative phytotoxicity.

2. Uramon, at rates as low as ¼ lb. per sq. yd., applied to light sandy soil heavily infested with *Heterodera marioni* in the fall prior to planting

in the following spring, usually resulted in increased growth and yields in comparison with those from nontreated plots. These increases were large enough to offset the residual deleterious effect of the Uramon treatment. In some experiments the addition of peanut-hull meal, cottonseed meal, manure, or a rye cover crop to plots treated with Uramon counteracted this deleterious effect upon certain crops.

3. On soil lightly infested with the root-knot nematode, applications of ethylene dibromide at 200, 400, and 600 lb. per acre in the fall or spring, of D-D and chloropicrin at 200 and 400 lb. in the fall or of chloropicrin at 200 lb. in the spring appeared to be noninjurious to snap bean. D-D and chloropicrin applied in the fall at 600 lb. or in the spring at 400 and 600 lb. per acre resulted in marked reductions in yield and growth of snap bean.

PLANT PATHOLOGY SECTION
    NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION
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PHYTOPATHOLOGICAL NOTES

A Witch's-Broom Disease on Cucumber and Canteloupe.—During the latter part of the recent war the author was employed by the Foreign Economic Administration in connection with its program of vegetable production for the armed forces on certain of the islands in the South Pacific area. On Espiritu Santo in the New Hebrides group an unusual and interesting disease,[1] presumably caused by a virus, was observed on plantings of canteloupe (*Cucumis Melo*) var. P.M.R. No. 45, and cucumber (*C. sativus*) var. Long Green. So far as can be determined this disease has not been previously described. Circumstances did not permit experimenting, so that only a description of symptoms was obtained. The symptoms described are those observed on cucumber; those on canteloupe were essentially the same.

General symptoms were of a "witch's-broom" type. The infected portions of the plants appeared as a mass made up of numerous flowers and undersized leaves. In early infection the whole plant was much reduced in size, showing typical symptoms throughout; in later infection only subsequent growth was affected. Mosaic and other pattern type symptoms were absent. The infected portions were uniformly green, slightly paler than normal, with spindly stems. Infected plants remained alive and growing until removed by cultural operations.

Internodes were somewhat shortened, and nodes in contact with moist soil readily produced adventitious roots. On growth subsequent to infection, branching occurred at every node of the primary stems. Every node on both primary and secondary stems produced from three to six flower buds, usually only one flower opening at a time. The shortened internodes, excessive branching with leaves at each node, and profuse flower production gave the characteristic dense "witch's-broom" appearance.

Leaves, though undersized, were nearly normal in shape. Petioles were seldom more than 2 in. long, blades not more than 2½ in. long by 1½ in. wide, often smaller. Flowers were abnormal and nonfunctional. Pedicels were 1 to 1½ in. long, very slender and weak. Petals of open flowers were approximately ½ normal size, yellow to greenish-yellow.

The proportion of male to female flowers was far in excess of the usual. Male flowers had definitely enlarged sepals which were either narrow and straplike, up to ½ in. long, or more often leaflike with a definite petiole and lanceolate to pointed ovate blade up to ⅝ by ¼ in. Female flowers had normal-shaped sepals proportionate in size to the petals. Ovaries at time of blossoming were about ⅝ in. long, more slender than usual and with

[1] Early in March, 1949, Dr. O. A. Lorenz observed the same or a very similar disease causing extensive damage on commercial plantings of cucumbers near La Paz, Baja California, Mexico. The presence of a new disease of this type so close to production areas in the United States is noteworthy.

spines much reduced. Sometimes developing to 1–1½ in., the ovaries always turned yellow and dropped, as did fruit partially grown before infection occurred.

No other cultivated cucurbits than cucumber and canteloupe were available for study during the period when the disease was observed. A *Momordica sp.* which grew profusely everywhere on the island may have been a symptomless carrier, but the definite seasonal occurrence noted below would be unlikely with an infected host in close proximity to the cultivated crops. An unidentified, much less common cucurbit which had similar symptoms may have been the source of the disease. Since the farming area was bordered on three sides by thick jungle growth, other host plants may have been present. The vector was not observed.

Although cucumbers were grown continuously throughout the year, the disease was observed only during December, January, and February, 1944–45. These summer months, on Espiritu Santo, are considerably less rainy than the rest of the year, and in this particular season virtually no rain fell during the months named, while the remainder of that year was extremely wet. Thus, considering the great variations in the island's rainfall during the year, together with the small annual range in mean temperature, it seems probable that the incidence of the disease is related to the former. The heavy rainfall during most of the year may have served to keep the presumed insect vector in check.—PAUL G. SMITH, Division of Truck Crops, University of California, Davis, California.

Asteroid Spot of Peach in North Carolina.—Leaves of three Indian Chief peach trees in a variety orchard of the Agricultural Experiment Station at Eagle Springs, North Carolina, had symptoms in July, 1946, resembling those of some virus diseases. These trees had been obtained from the Wolfe Nursery, Stephenville, Texas, and planted adjacent to each other in March, 1945.

Symptoms of the disease were similar in many respects to those of asteroid spot.[1] Affected leaves were rather uniformly stippled with numerous small, irregularly shaped, yellow or yellowish-green areas. Only leaf symptoms were noted. No breaking of the blossoms, delay in foliation, premature defoliation, reduction in growth, or malformation of leaves, fruit, or flowers have been observed. The leaf symptoms are imperceptible early in the season, but become rather conspicuous with the advent of high temperatures towards mid-season, and remain distinct on fully expanded leaves until they drop in the fall.

The disease has been found to be bud-transmissible. Buds from affected trees were inserted on September 6, 1946, into four J. H. Hale and one Golden Jubilee peach tree growing in 10-in. pots under glass. All buds united with the stocks but shoots grew from only three of them. The leaves

[1] Cochran, L. C., and Clayton O. Smith. Asteroid spot, a new virosis of the peach. Phytopath. **28**: 278–281. 1938.

on these shoots and on branches below the point of budding had typical symptoms in June, 1947. On June 6, 1947, buds from affected Indian Chief trees were placed in seven potted Golden Jubilee trees. Leaf symptoms were apparent on shoots below the point of budding by August 21, 1947. In another test started in September, 1947, buds from affected Indian Chief trees were placed into 16 potted trees including one or more of 14 varieties. All buds united with the stocks. In 1948, shoots grew from six of the buds. Typical symptoms developed by midsummer on the leaves of these Indian Chief shoots. The leaves of other shoots on the 16 budded trees also showed typical symptoms, being more conspicuous for some varieties than for others. Figure 1 shows a J. H. Hale leaf from a branch growing above an
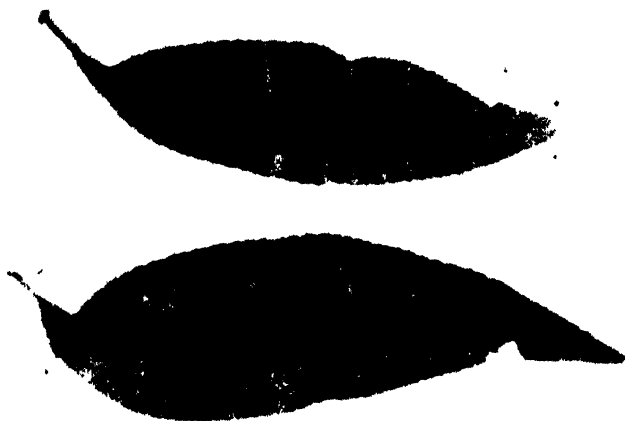
FIG. 1. Peach leaves with symptoms of asteroid spot. Upper, from a J. H. Hale shoot that grew above an inserted Indian Chief bud. Lower, an affected Indian Chief leaf.

inserted bud, in comparison with one that grew from an inserted Indian Chief bud. No symptoms appeared in the controls.

Examination of specimens and photographs of affected leaves kindly sent by L. C. Cochran[2] supports the evidence that the three Indian Chief peach trees are affected by the virus disease known as asteroid spot.—C. N. CLAYTON, Plant Pathology Section, Agricultural Experiment Station, Raleigh, North Carolina.

Leaf Distortion in Sunflower Produced by Lanolin.—Lanolin paste has been used in various scientific investigations involving introduction of exceedingly small amounts of chemicals into plant cells and tissues. Since the time of Laibach[1] plant physiologists have been using it in plant hormone work, and geneticists as well as horticulturists have used it in their

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[1] Laibach, F. Versuche mit Wuchstoffpaste. Ber. Deut. Bot. Ges. 51: 386–392. 1933.

[2] Letter of November 18, 1948.

work in connection with the colchicine treatment of experimental materials. However, no report of any anatomical and morphological abnormalities attributable to lanolin itself in plants has been found by the writer.

Sunflower plants, of the variety Russian Mammoth, were grown in 6-in. pots in a warm greenhouse. When the first set of leaves was well de-
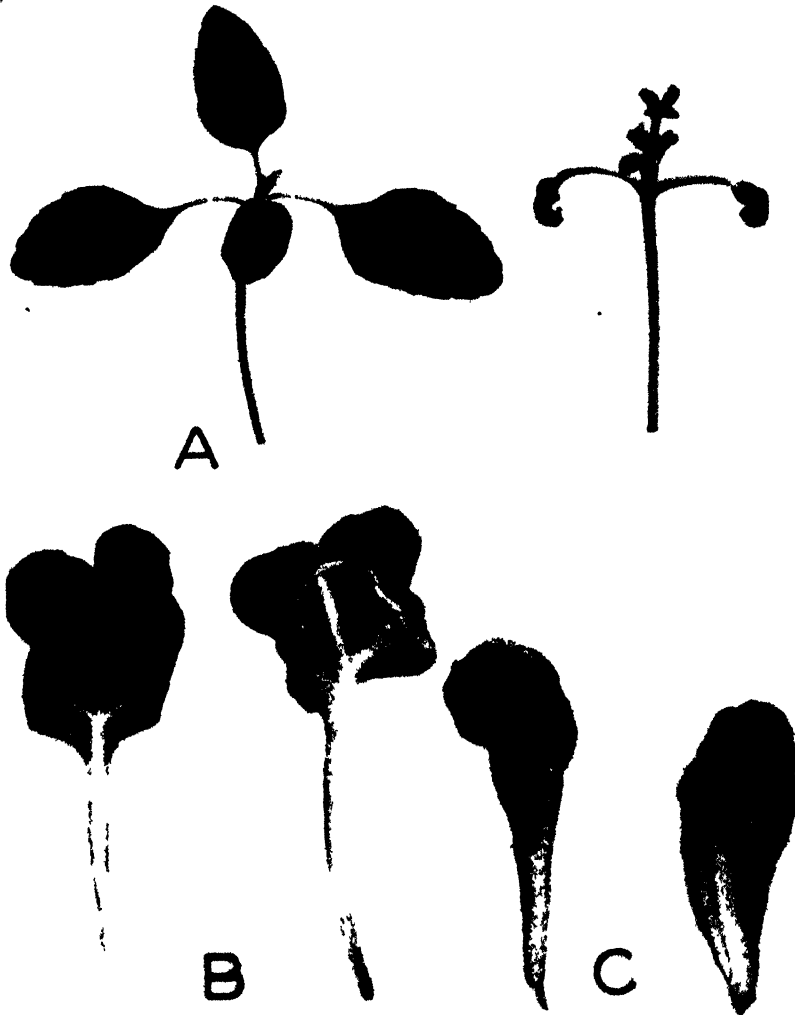
FIG. 1 Effect of lanolin on sunflower leaves   A. Left, check plant, treated with distilled water; right, plant with lanolin treated cut surface. B, C. Dorsal and ventral views of the leaves from a lanolin treated plant.

veloped, the growing point was removed with a sharp scalpel and a small amount of lanolin was placed on the cut surface with a glass rod. The lanolin used was U.S.P. grade, anhydrous, manufactured by Mallinckrodt Chemical Works. The checks consisted of sunflower plants with growing points removed, the wound treated with a drop of distilled water.

The plants were left on the bench in the same room. In a week or 10 days axial buds usually commenced to grow and were quite noticeable. On plants treated with a drop of water the leaves were normal while in lanolin-treated plants the leaves were underdeveloped and distorted in various ways (Fig. 1). This condition persisted for some time indicating a possible introduction of something into the plant from the lanolin. The morphological appearance of the sunflower leaves was somewhat like that of certain virus diseases and also was somewhat similar to injury by certain new herbicides such as 2,4-D. There is considerable reduction in growth as a result of the lanolin treatment. Distorted leaves develop very slowly and become brittle, breaking off easily on slight pressure. The phenomenon was not observed in tomato plants used at the same time in parallel experiments; hence it seems there is a certain degree of specificity.—PETER A. ARK, Division of Plant Pathology, University of California, Berkeley, California.

ANNOUNCEMENT

SEVENTH INTERNATIONAL BOTANICAL CONGRESS
STOCKHOLM—1950

The Seventh International Botanical Congress will be held in Stockholm, Sweden, between July 12 and 20, 1950. Presessional excursions will start on June 27 and postsessional excursions will end on August 9. Communication No. 2 from the Organizing Committee, with the preliminary outline of the program during the meeting and the preliminary plans for excursions, may be obtained from the Secretary General, Dr. Ewert Åberg, Uppsala 7, Sweden.

THE EFFECT OF PARTICLE SIZE AND SOLUBILITY OF SULFUR IN CARBON DISULFIDE UPON ITS TOXICITY TO FUNGI

Edmund F. Feichtmeir

INTRODUCTION

Despite the commercial production of new synthetic fungicides, sulfur continues to hold an important place among materials used for plant protection. In many respects its action is but poorly understood and hence further experimental work seemed worthwhile. In particular, attention has been given to the relative fungicidal potency of different allotropic forms and to the effect of variation in the method of preparation. Since it has long been known that the effect of sulfur preparations is related to the size of particles (18, 21), special effort was made to prepare experimental samples of very homogeneous characteristics.

There are several allotropic forms of sulfur, some crystalline, others amorphous (8, pp. 236-237). All of the crystalline forms are soluble in carbon disulfide (CS_2), whereas there are both CS_2-soluble and CS_2-insoluble amorphous forms. At normal temperatures all of the crystalline and CS_2-soluble amorphous forms transform to the rhombic state in two or three days (8). The CS_2-insoluble amorphous form remains rather stable. This difference in solubility in CS_2 was utilized in this study for comparing the toxicity toward fungi of allotropic forms of sulfur.

MATERIALS AND METHODS

Size ranges of 1-2μ, 5-6μ, 10-11μ, and 15-16μ diameter were separated by liquid sedimentation according to the principle of Stokes' Law of falling bodies (17). Two-liter cylinders were used as settling chambers for the large fractions, and oblong, flat-bottomed glass containers approximately 9 inches × 10 inches and 8 inches high for the smaller sizes. A thick suspension was made by adding sulfur to a solution of polyether alcohol (Triton X-100) in water and mixing in a Waring Blendor. This suspension was poured into the 2-liter cylinder, made up to volume, and mixed by inverting back and forth for 1 min. The final concentration of alcohol was 0.1 per cent. The cylinder was then placed upright and the time immediately noted. The time required for all particles larger than the maximum of the cut being made to settle the distance from the 2-liter mark (A) to the 1-liter mark (B), was calculated. When this time had elapsed all the liquid above B was withdrawn by gentle suction on a small orificed pipette or siphon. This mixture was then placed in another cylinder, brought up to 2 liters with 0.1 per cent alcohol solution, and the process was repeated, except that the time required for the finest particles of the cut to settle a distance from A to

605

B was used. The liquid above B was again withdrawn but this time was discarded. The fraction below B contained a mixture of particles none of which were larger than the upper limit as well as some particles smaller than the minimum size desired. By repeating the settling process 5 to 10 times, depending upon the particle size of the cut desired, those particles smaller than the lower limit were removed and a very uniform, narrow cut obtained.

Microscopic examination showed the 1–2 μ samples used in the tests to be mostly 2 μ in diameter. The other ranges were of uniform distribution although the larger sizes had a greater variability in shape than the smaller ones.

The alcohol was slightly toxic to some of the test organisms at the concentrations necessary to keep the particles dispersed; so, following the final separations, the alcohol was removed by the process of centrifuging, decanting, and replacement with a 0.05 per cent solution of blood albumin. Blood albumin solution was not used as the original dispersing agent because at room temperature there was a tendency for some putrefaction to occur during separation of the smallest particle size range. However, the final samples were stored at 4° C. with no detectable putrefaction.

Even when using blood albumin or polyether alcohol, which were the best of all dispersing agents examined for use with sulfur, only a relatively small percentage of the original samples was dispersed into individual particles.

The CS_2-insoluble sulfur was obtained by leaching a commercial sublimed sulfur (approximately 35 per cent of the insoluble form) with CS_2. The sublimed sulfur was allowed to stand in the solvent for 5 min., then was filtered and washed continuously for 1 hr., using 2 gal. of CS_2 for 1000 gm. of sulfur. Following washing, suction was applied to remove as much CS_2 as possible. The sample then was spread out in a fine layer on filter paper and allowed to dry for 24 hr. At this stage an average particle size of 2.2 μ was obtained by Gooden and Smith's air permeability method (6). Microscopic examination indicated that what appeared to be much larger particles were actually agglomerates which partially dispersed because of the action of immersion oil. Since practically all of the individual particles were 3 μ or smaller in diameter, only the 1–2 μ fraction was separated for this form; no CS_2 could be detected in these separations. Although many attempts were made to produce CS_2-insoluble sulfur of larger particles so that comparisons could be run over a range of sizes, none was successful.

The CS_2-soluble fractions were from mined sulfur either ground as such or with the addition of 0.5 per cent magnesium carbonate. Magnesium carbonate is non-toxic at concentrations which might be present. The micronized sulfur used, also CS_2-soluble, was that sold under the name "Butcher Brand"; its average particle size was 5 μ but no difficulty was encountered in obtaining samples for all ranges between 1 μ and 16 μ diameter. Since some of the above samples had a small amount of the CS_2-insoluble fraction

present, they were dried at 95° C. for 48 hr. to render them soluble before separating the size ranges. After this treatment analysis showed the samples to be completely soluble in CS_2.

The quantity of sulfur present in the final samples was determined by Sommer's pyridine method (15). The dried sample required for this analysis was obtained by evaporating an aliquot of the sulfur suspension with the aid of suction from a water aspirator.

Four organisms and three techniques for assaying toxicity were used. The first method, drop-dilution, was similar to McCallan and Wilcoxon's (10) test-tube dilution slide-germination test, with conidia of *Sclerotinia fructicola* (Winter) Rehm being used as the test organism. The technique in this study differed from McCallan and Wilcoxon's method in that no stimulant other than blood albumin was added; and further, the spore suspensions were obtained by washing conidia from the culture slants with 0.05 per cent blood albumin solution by a gentle rotation of the tube between the hands. In addition, Petri dishes were employed as moist chambers with one test slide per dish.

The second method using the barley mildew fungus was employed for comparative purposes. In utilizing this method, three drops of sulfur suspension were placed on a slide and allowed to dry. Then the slides were placed at the bottom of a settling chamber and dusted with the conidia of *Erysiphe graminis hordei* Marchal from an 8-day culture on potted barley plants. The dry slides were placed in Petri dishes without a water seal and incubated 20 hr. at 16° C. The relative humidity of the incubator was 9 per cent by the wet bulb method. The number of spores counted for each treatment was equal to that required to give 100 germinating spores on the control. Since the controls consistently yielded low germination values (approximately 50 per cent), several were run in each experiment. A difference of plus or minus 2 per cent germination for these controls was adopted as the maximum allowable experimental variation for which data would be considered reliable.

The bean rust fungus, *Uromyces appendiculatus* (Pers.) Lev., and the bean mildew organism, *Erysiphe polygoni* D C, were used as test fungi in the third method. Pinto bean plants 10–12 days old were used for inoculation. They were pinched back to a single leaf because of the limited quantity of the sulfur samples. Preliminary experiments showed that 1.5 ml. of suspension could be sprayed on an average leaf before runoff commenced. A De Vilbiss hand sprayer was modified so that it could be used on a compressed air line. A vial containing 1.5 ml. of sulfur suspension was made up for each leaf surface. Spraying was done in the morning and the leaves were allowed to dry until 5 o'clock in the afternoon, when they were inoculated by spraying with spore suspensions and placed in a moist chamber overnight. They were then placed upon a bench in the greenhouse and a period of 10 days was allowed for the rust pustules to develop after which the number per square centimeter was counted. The mildew infection was

estimated 11 days after inoculation on a 0–10 basis. The value of 10 was given to the control in each experiment; zero indicated there was no infection.

The data were plotted on log-probability paper with straight lines being used to represent the best dosage-mortality curves (2). Since in nearly all of the experiments in this study the slopes of the lines within each test were essentially identical, it would make little difference what LD level was chosen for comparative purposes. According to McCallan and Wilcoxon (11), LD 50 is the most precise point for comparing compounds of essentially similar slopes. Most of the points for the curves fell between 5 per cent and 95 per cent; so for uniformity the 50 per cent level was used throughout.

OBSERVATIONS AND EXPERIMENTAL RESULTS

In figure 1 are shown the comparative toxicities of CS_2-insoluble and
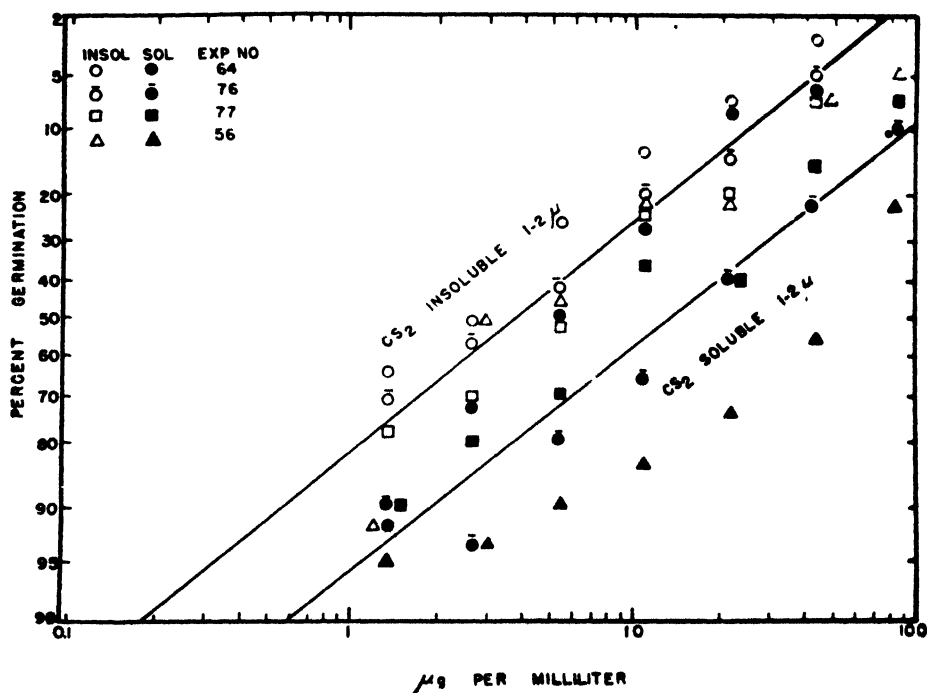
FIG. 1. Toxicity for conidia of *Sclerotinia fructicola* of CS_2-insoluble and CS_2-soluble sulfur with particles 1 to 2μ in diameter.

CS_2-soluble sulfur at the 1–2 μ diameter size against the conidia of *Sclerotinia fructicola*. Regression lines were calculated for best fit. They are practically parallel and no doubt would be if more points had been obtained, indicating that the toxicity of the two forms is due to the same causative agent.

The results of experiments in which 1–2 μ diameter size ranges of CS_2-

insoluble and CS₂-soluble sulfur were compared for toxicity against conidia of *Erysiphe graminis hordei* are shown in figure 2.

With sulfur particles 1–2 μ in diameter the concentrations of CS₂-insoluble and CS₂-soluble sulfur, respectively, in μg per ml. for 50 per cent reduction in germination or infection were as follows: for germination of conidia of *Sclerotinia fructicola*, 3.19 and 13; for germination of *Erysiphe graminis hordei*, 0.6 and 2.1; for protection against *Uromyces appendiculatus*, 30 and 80; and for protection against *Erysiphe polygoni*, 1.9 and 12.
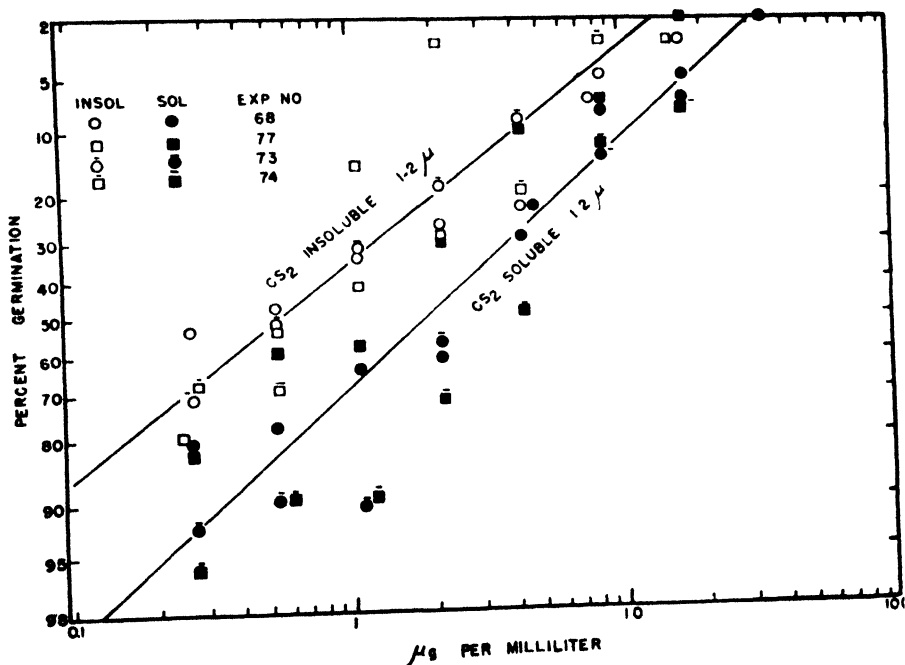
FIG. 2. Toxicity to conidia of *Erysiphe graminis hordei* of CS_insoluble and CS₂-soluble sulfur with particles 1 to 2μ in diameter.

The factor of particle size was investigated for two reasons: the first was to obtain more precise information concerning the effect of particle size on toxicity; the second was to resolve if possible the differences between the findings of Persing *et al.* (13), and those of Wilcoxon and McCallan (21), Hamilton *et al.* (7), and others. The latter group has published numerous data to show that a decrease in particle size increases toxicity. Persing, however, found that against thrips a medium particle size range, 30–40 μ, was more effective.

Method of grinding was briefly investigated along with the particle size experiments, because Alexander (1), working with inert insecticides, has shown this to be a factor which might affect toxicity. The claim has been made, apparently without substantiation, that at the same average particle size, micronized (12) sulfur is more effective than corresponding Raymond Mill ground material. In the micronizing process air at 100 lb. pressure is

used to propel particles against one another causing them to be shattered and thus reduced in size. No conclusive scientific evidence has been found in the literature which substantiates or refutes any differences in sulfur toxicity due to method of grinding.

In this study the relationship of toxicity to particle size using CS_2-soluble sulfur of 1–2 μ, 5–6 μ, 10–11 μ, and 15–16 μ diameter sizes is shown in figures 3 and 4. Figure 3 shows the effect of concentration on germination of
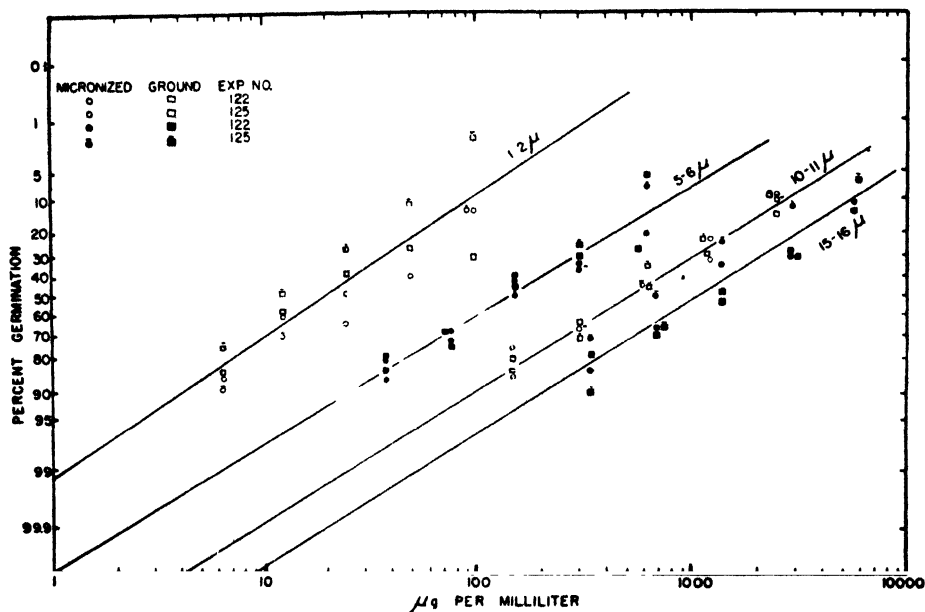
FIG. 3. The effect of particle size of CS_2-soluble sulfur on toxicity to conidia of *Sclerotinia fructicola*.

conidia of *Sclerotinia fructicola*. Micronized and regular ground sulfur showed no noticeable difference in toxicity; two tests of each are plotted together in figure 3. By dividing the LD 50's in figure 3 by the LD 50 of the 1–2 μ range the following ratio is obtained: 1 : 7.4 : 24.6 : 55. It is important to note that by using the square of the radius of each particle size, a very similar ratio is obtained: 1 : 6.3 : 25 : 56. This ratio was calculated on the basis of the observation that the smallest fraction was practically all 1 μ in radius.

The effect of concentration on germination of the conidia of *Erysiphe graminis hordei* for the four size ranges is shown in figure 4. Data from the tests with the other two organisms corroborated the findings in figures 3 and 4.

During the work with bean rust the plants stood on a bench in the greenhouse and were exposed to air-borne infection by bean mildew. This gave an opportunity to evaluate the residual effect of the different particle size preparations. When the degree of mildew infection after two and three weeks was compared at the concentration for each particle size at which 95 per cent protection against rust was obtained, a large amount of

infection was found in the 1–2 μ range, a very small amount in the 5–6 μ range, and none in the larger size ranges. Based on the time usually required to reach the state at which readings were made, one would estimate the period of protection as less than one week for the 1–2 μ size and more than a week for the 10–11 μ and 15–16 μ ranges. These observations confirm the findings of Persing *et al.* (13) using average particle size.
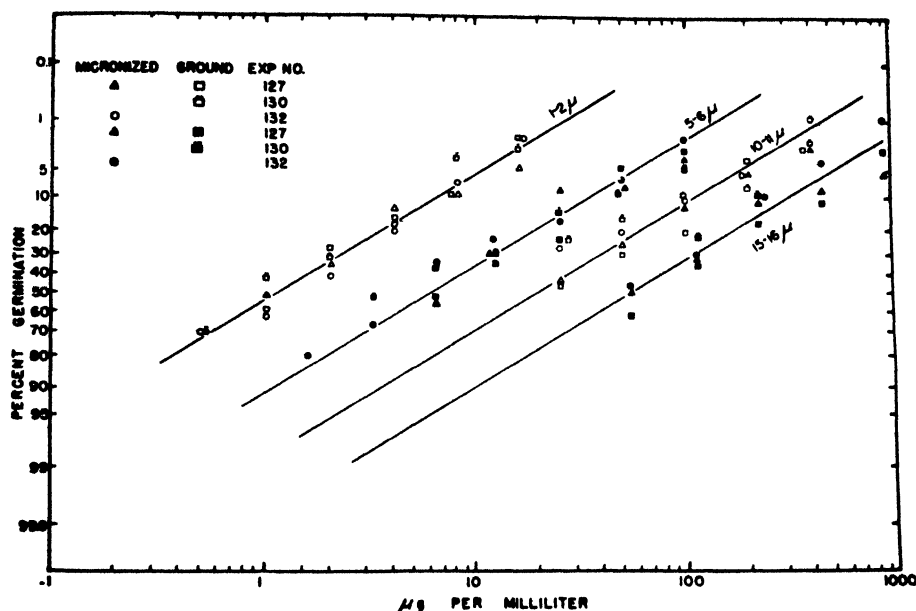
Fig. 4. The effect of particle size of CS₂ soluble sulfur on toxicity to conidia of *Erysiphe graminis hordei*.

As previously noted, CS₂-insoluble sulfur has a very strong tendency to form agglomerates. While attempting to reduce the concentration of the polyether alcohol by centrifuging, decanting, and adding water in an aliquot of the 1–2 μ fraction, agglomeration commenced. Further dilution with water was stopped and 0 05 per cent blood albumin solution was used instead. Part of the sample remained agglomerated. Another aliquot of the same original suspension was washed an equal number of times with 0.05 per cent blood albumin solution. All the particles in both samples were the same size, 1–2 μ, but one sample was completely dispersed while the other had an appreciable number of agglomerates. Using the drop-dilution technique the concentration required to prevent germination of 50 per cent of the conidia of *Sclerotinia fructicola* was determined for each sample. The results showed that the non-clumped material was four times as toxic as the agglomerated sample. These data indicated that further studies of agglomeration effects might be profitable.

Some exploratory toxicity tests were run with amorphous sulfur which was obtained by rapid evaporation of a measured amount of an acetone solution of sulfur upon 15-mm. cover slips. The cover slips were secured to

slides by the attraction of a film of water between the two surfaces. The *acetone solution of sulfur did not run over the edges of the cover slips.* Microscopic examination showed that the particles remaining after evaporation of the acetone were mostly 1–2 μ in diameter. Although there were possibly many particles outside the range of resolution, most of the mass was of the larger size. Conidia of *Sclerotinia fructicola* in 0.05 per cent blood albumin solution were used in testing toxicity; they were placed upon the cover slips within 4 hr. after the particles were formed, and the standard procedure, as outlined for the drop-dilution method, was followed. The amorphous sulfur was more than 10 times as toxic as the CS_2-soluble form, requiring only 0.13 μg per sq. cm. to give 50 per cent inhibition.

Although it is not justifiable to conclude that the above figures give the true relative toxicities, since the samples could not be separated into definite particle size ranges because of the rapid rate of transformation to the crystalline form, an LD 50 of 0.13 μg per sq. cm. for *Sclerotinia fructicola* still indicates remarkable toxicity for sulfur.   .

DISCUSSION

The data for the CS_2-insoluble and CS_2-soluble sulfurs showed the former to have about three times the innate toxicity of the latter. It does not mean, however, that commercial sulfur of the former type would have three times the toxicity in the field. Branas's data (3) indicated that sublimed sulfur containing approximately 35 per cent of the CS_2-insoluble form was not appreciably more toxic than crystalline sulfur toward grape mildew, *Uncinula necator* (Schw.) Burr. The CS_2-insoluble sulfur is composed principally of spherical particles grouped together like a bunch of grapes and difficult to disperse. From the data on the effect of agglomeration one might expect that the higher innate toxicity of the CS_2-insoluble sulfur would be counteracted by the formation of agglomerates.

Branas (4) has found essentially the same effect of agglomeration by chemical methods that is shown in this study by biological means. Using aliquots of the same sulfur sample, he showed that as the weight per unit area increased the vapor given off per unit weight decreased.

It is interesting to note in connection with the use of blood albumin and glue as dispersing agents, that Yothers and Miller (22) and Stafford (16) found these two materials very effective for increasing deposit and adherence of sulfur on citrus and apples, respectively. It is well known that the smaller the particle, the greater is its sticking power. Blood albumin was, no doubt, breaking the agglomerates. The argument that increased fungicidal effect was due entirely to heavier deposit ignores the effect of albumin and glue in decreasing agglomeration. In the light of the toxicity data on clumping one should also expect greater toxicity due to greater dispersion even though resistance to weathering was not improved.

The increase in toxicity upon decreasing particle size was clearly demonstrated. It does not seem probable that the high correlation of toxicity

with surface area in the *Sclerotinia fructicola* tests could have been pure chance, for the general relationship throughout is too pronounced. It is well known that it is not necessary for sulfur particles to touch the spores in order to kill. The amount or rate at which sulfur vapor is given off per unit weight must have a high correlation with toxicity.

Apparently, method of grinding has little or no effect upon innate toxicity of sulfur. No significant difference in toxicity of sulfur against four organisms tested at four particle size ranges was shown between micronized and Raymond Mill ground sulfur in this study.

The low concentrations at which control was reached for the various samples indicate the possibilities of sulfur as a toxicant when well dispersed and of small particle size. This evidence together with the remarkable toxicities shown by sulfur obtained from acetone solutions offers a possible explanation for the great toxicities found by Liming and Young (9), Roach and Glynne (14), and Tisdale (19). Most of the experiments by these workers were conducive to the production of extremely fine amorphous sulfur and this rather than pentathionic or thiosulfuric acid probably accounted for their results. Tisdale states that his paste, made from the reaction of sulfur dioxide with hydrogen sulfide, lost its toxicity upon crystallization. This also indicates that he may have been dealing with an amorphous form.

From the above discussion it might appear that the smaller sizes should always be the most effective fractions. This is normally true but there may be apparent exceptions. As previously noted, when the particles are agglomerated toxicity is reduced. Also, when sulfur must retain its action over a long period of time, as is required in control of thrips (13), this may not apply. The smaller particle sizes do not weather well. The rust and mildew experiments demonstrated this, as did Persing's work. Weathering in these cases does not involve withstanding rains. Temperature is the important factor, especially the temperature of the leaves or fruit upon which the sulfur is placed. The findings of Curtis (5), who showed that it is possible for leaf temperatures to be 2° to 10° C. higher than the surrounding air temperature, and Turrell *et al.* (20) who found rind temperature of oranges to average 23° F. higher than air temperature, are worth considering in this connection.

One may conclude from the observations and experimental results in this study that the innate toxicity of sulfur is increased by any factor, such as favorable allotropic form (*i.e.*, CS_2-insoluble; amorphous from acetone solution), higher temperature, smaller particle size, or increased dispersion, which causes a greater amount or rate of production of sulfur vapor per unit weight of sample.

SUMMARY

The influence of sulfur form, particle size, and type of grinding upon toxicity against fungi has been investigated using particle size ranges of

1–2 μ, 5–6 μ, 11–12 μ, and 15–16 μ diameters plus or minus one micron. These narrow size ranges were separated by means of liquid sedimentation.

With sulfur particles 1–2 μ in diameter the concentrations of carbon disulfide-insoluble and carbon disulfide-soluble sulfur, respectively, in μg. per ml. for 50 per cent reduction in germination or infection were as follows: for germination of conidia of *Sclerotinia fructicola*, 3.9 and 13; for germination of conidia of *Erysiphe graminis hordei*, 0.6 and 2.1; for protection against *Uromyces appendiculatus*, 30 and 80; and for protection against *Erysiphe polygoni*, 1.9 and 12.

The LD 50 against conidia of *Sclerotinia fructicola* for carbon disulfide-soluble sulfur particles of 1–2 μ, 5–6 μ, 10–11 μ, and 15–16 μ in diameter decreased directly as the surface area per unit weight increased.

The agglomeration of individual sulfur particles decreased their fungicidal effectiveness.

Large particles retained their protective ability for a longer time than did small ones.

Micronized sulfur showed no greater innate toxicity than Raymond Mill ground sulfur.

In addition, an amorphous form (particles approximately 2 μ in diameter) produced by evaporating an acetone solution of sulfur gave a high toxicity against conidia of *Sclerotinia fructicola*—LD 50 of 0.13 μg. per square centimeter.

SHELL OIL COMPANY

MODESTO, CALIFORNIA

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A GALL OF TAHITI LIME AND OTHER CITRUS SPECIES CAUSED BY DODDER

L. CARL KNORR

INTRODUCTION

Excrescences on the bark of citrus are of various types and etiologies. One of the most common is Sphaeropsis knot caused by the pathogen *Sphaeropsis tumefaciens* Hedges (6). Fawcett (3) describes other abnormal growths resembling those produced when *Agrobacterium tumefaciens* (Smith and Townsend) Conn was inoculated into citrus species, and refers (2) to J. C. Perry's classification of citrus galls into three types: (a) those having a rough, more or less spongy surface, (b) those covered with scaly, hard-pointed projections, and (c) those covered with a smooth bark similar to that of the trunk or limb and consisting of a hard woody tissue underneath. Peculiar burrlike growths bearing numerous buds are described by Shamel, Pomeroy, and Caryl (8) as occurring in the leaf axils of Washington navel oranges; these, however, failed to show any diseased condition, and were regarded as being heritable. "Knobby bark" is described by Fawcett and Bitancourt (4), and the galls are deemed a possible virus effect which may be related to psorosis. Nowell (7) mentions the sporadic occurrence in St. Lucia and Dominica of citrus galls in which no organisms could be found; and Savastano, cited by Fawcett (2), also refers to protuberances of a similar type in Sicily.

Galls on citrus differing from those hitherto described are reported here. Examples were found in a grove of Tahiti lime (*Citrus aurantifolia* (Christm.) Swingle) in Winter Haven, Florida, during the summer of 1948. At first glance these appeared to be Sphaeropsis knot, but further observation showed the galls to be arranged in a dextrorse spiral; and this right-handed ascendancy, reminiscent of the way dodder twines, furnished a clue as to their possible origin.

SYMPTOMATOLOGY

Morphologic symptoms.—Figure 1, A, B, and C,[1] from left to right, shows a gradation in age and severity of dodder galls. On recently attacked stems the spiral pattern of minute, callused cankers left by the dead haustoria readily suggests their dodder origin. Older galls, however, present a less evident aspect of such origin, and it is these which at first sight may be confused with Sphaeropsis knot. Here again, however, the dextrorse arrangement can be traced without much difficulty and the identification facilitated.

On stems of recent infection, as typified by figure 1, A, the dodder leaves a band of slightly raised, light-colored bark, and in this band are to be seen the regularly spaced oval craters, former sites of the haustoria, averaging 1 x 1.5 mm. in diameter. Other stems (Fig. 1, B) may not show a continuous band of abnormal bark but may consist of discrete light-colored, lenticular or circular craters ascending the twig in a spiral, with normal bark between the cankers. The oldest infections (Fig. 1, D to H)
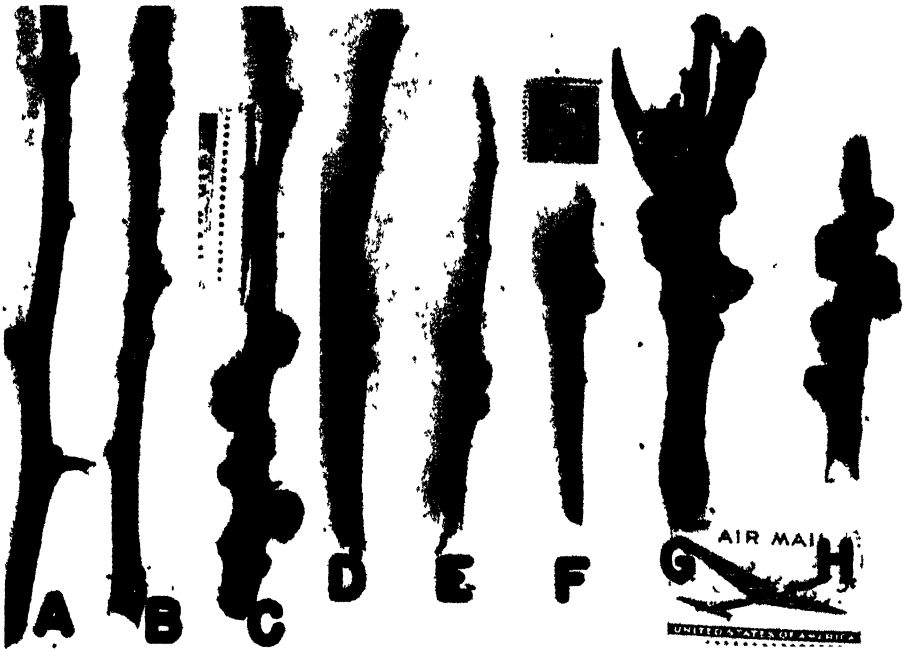
FIG. 1. Dodder galls on Tahiti lime. A, B, and C: Gradation in age and severity of galls. D, E, and F: Gall formation at nodes leading to a stunting of new wood. G and H: Typical aspect of galls, 163 of which occurred on 7 representative stems totaling 85 in. in length.

consist of tumors with hard woody outgrowths covered by bark that ranges from normal in color and consistency to that which is a lighter color and somewhat scaly. Shape of the fully developed galls is roughly globoid, with the equator averaging a third larger than the smallest meridian adjacent to the base. Occasionally the galls merge into one another and form a continuous ridge (Fig. 1, F and H). Characteristic of the causal dodder, whose zone of haustorial formation is generally limited to four circumnutations of the stem, these galls are rarely to be found with more than four decks per stem section. Most affected stems have eight galls per spiral revolution.

In orange (*Citrus sinensis* (L.) Osbeck) and grapefruit (*Citrus paradisi* Macf.), parasitism of the same species of dodder does not lead to any appreciable overgrowth, and symptoms are limited to craterlike processes similar to those shown in figure 1, A and B.

Approximately half of the twigs on a quarter of the lime tree worst affected showed these galls. Seven typical twigs were singled out for measurement and counting; these totaled 85 in. in length and bore 163 galls similar to those shown in figure 1, C, G, and H.

Histologic symptoms.—Cross sections of the older galls reveal a hypertrophy of the tissues overlying the cortex, and in the oldest galls no longer harboring the dodder, conical plugs of resinous material pierce the tumors to the cortex. Young galls, still the seat of active haustorization, show the intermingling of cell elements typical of dodder-suscept relationships.

<h2 style="text-align:center">ETIOLOGY</h2>

Tumor production of dodder has been reported in the past. Dean (1) lists 28 species of plants, none of them citrus, as being gall-forming hosts to one or more of five species of *Cuscuta*. Stout (9) describes a swelling of olive caused by *Cuscuta indecora*, and Graham (5) mentions galls 1 in. in diameter on the stems of *Hibiscus* and attributes them to the dodder *Cuscuta reflexa*. Fawcett (2), in the second illustration of his book on citrus diseases, shows two tubercles on a sour orange seedling, but makes no further comment other than to point out in the caption the gall-like swellings which he states are a later effect of previous dodder infection.

The dodder found in constant association with early stages of gall formation was *Cuscuta americana* L.[2] When haustorium-producing coils encircling the twigs were removed, craters appeared of a form and color already described. Dodder was found issuing from small and medium galls pictured in figure 1, B, but not from larger galls the size of those in figure 1, C to H.

Sections of gall tissue were examined microscopically and only in the younger galls were there to be seen any extracellular components—and these were but of the haustoria themselves. Older tumors (cf. figure 1, C to H) showed only plugs of resinous material in a matrix of hypertrophied twig-cell elements. No microorganisms were found in the galls regardless of their age.

While no inoculation tests with the dodder have been made, it does appear that gall production in this case is not so much a result of initial penetration of haustoria as of the periodic tearing out of aerial parts of the vine and the consequent metabolic acceleration that is involved in the vine's repeated attempts at restoration.

<h2 style="text-align:center">IMPORTANCE</h2>

The consequences of such dodder galls are twofold. First, they cause a distortion which, if the apical growth region is affected, leads to a stunted development of new wood (Fig. 1, D, E, and F). Second, and more important, these galls, particularly in their younger stages, are the seat of haustoria that have the ability to regenerate aerial dodder even after all

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[2] Determined through the courtesy of Dr. T. G. Yuncker, DePauw University.

external vines have been stripped from the tree. Through renewal of
growth in such a manner the dodder in this particular grove has survived
all efforts at eradication over the past five years, even when recurring
vine growth was cleaned out every 10 to 20 days throughout the rainy sea-
son.   Figure 2, A, shows the amount of aerial dodder developing in 11
days after previous growth had been removed from the tree and the point
of dodder attachment had been scraped by fingernail so that it was flush
with the bark of the twig. Commercial removal of dodder, such as that
obtained when the crew "cleaned up" a tree, resulted within 20 days in
the amount of aerial dodder shown in figure 2, B.
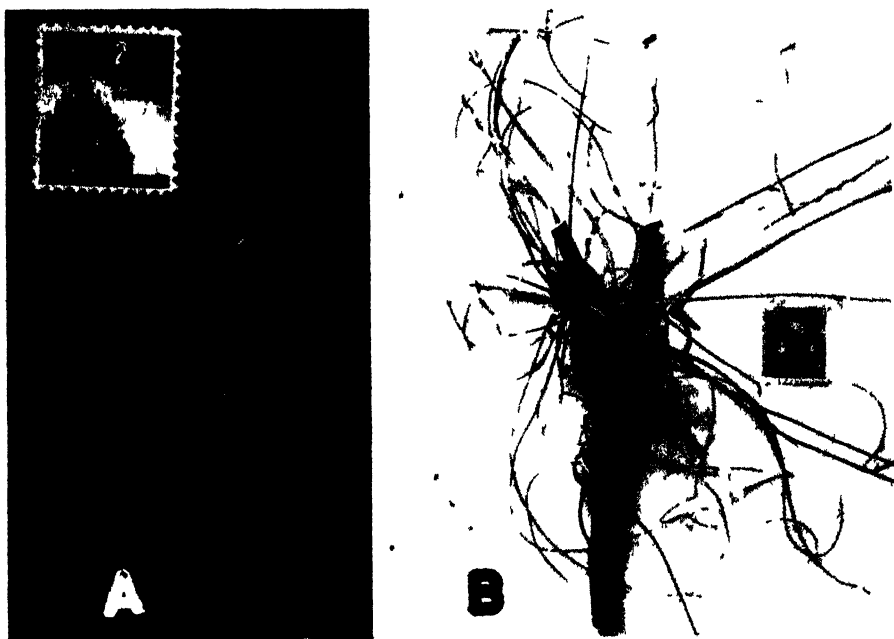
FIG 2. A. Amount of aerial dodder developed in 11 days after previous growth
had been removed from tree and point of dodder attachment had been scraped by
fingernail flush with bark of twig   B Twenty day old regrowth of dodder after com-
mercial eradication by labor crew.

These infective tumors, capable of regenerating aerial dodder from
haustorial fragments, have been noticed only on Tahiti lime, and have
not to date been found on some 70 orange and grapefruit trees infected
by the same dodder. This observation is in agreement with the experi-
ence of growers who report the possibility of eradicating dodder by hand
completely from orange and grapefruit trees, but not from lime.

<div style="text-align:center">CONTROL</div>

Every 20 days throughout the rainy season, from June to October, a
crew of laborers spends a day in this grove eradicating dodder which has
grown since the last removal. This has been going on for the past five

years, and according to the caretaker, the infestation appears to be getting worse. The removal is being done carefully, even down to the rubbing off of the tenacious haustorial coils, but the submerged haustoria are negating all such attempts at control. Obviously a better method of eradicating this infection would be to prune out the infected wood or to cut back the tree and, since *Cuscuta americana* can reproduce by seed, to cultivate the ground underneath so as to preclude reinfection by next spring's seedlings.

SUMMARY

A gall of the branches of Tahiti lime, similar to that caused by *Sphaeropsis tumefaciens*, is described and its formation attributed to *Cuscuta americana* L. Such dodder galls lead to a stunting of twig growth and are the seat of haustoria that remain to regenerate aerial vines after the dodder has ostensibly been eradicated. Control of dodder in limes necessitates the pruning out of infected wood or the hatracking of the tree.

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STUDIES ON VARIABILITY OF SCLEROTINIA FRUCTICOLA (WINT.) REHM[1]

K. S. Thind and G. W. Keitt

INTRODUCTION

Within recent years it has been increasingly apparent that certain types of phytopathogenic fungi offer very favorable material for genetic and physiological studies relating to basic problems of parasitism and disease resistance (4, 10). *Sclerotinia fructicola* (Wint.) Rehm, the common brown rot fungus of stone fruits in North America, seems to have potential suitability for such work; but, in spite of a substantial amount of valuable research on this organism, there are still important gaps in the knowledge of its cycle of development. From the available literature it would appear probable that combinations and segregations of genetic factors occur in the development of its ascigerous stage, but the evidence on this important point is meager and methods for experimental crossing of isolates of the fungus are lacking. The chief purposes of the present investigation were to contribute to the knowledge of the cycle of development of this pathogen and to explore some possibilities of its being suitable for basic studies. A portion of the work dealing primarily with nutrition of the fungus is reserved for publication elsewhere.

Variations within the American species of brown rot fungus of stone fruits were reported by Reade (6), but he stated that these differences disappeared when the strains were cultured under uniform conditions. Ezekiel (3) showed conclusively that strains of *Sclerotinia americana* (Wormald) Norton and Ezekiel (Syn: *Sclerotinia fructicola* (Wint.) Rehm) collected from Maryland and other parts of United States differ widely in their characteristics in culture or on rotting fruits and in oxidase production, size of conidia, *etc.*, and that these are genetic variations rather than modifications due to environment. Ezekiel grouped more than 30 strains of *S. americana* into 6 varieties differentiated by their habit of growth on potato-dextrose agar at 25° C. in tube cultures and at 15° C. in plate cultures. These varieties retained their differences under widely different environments and after successive passages through artificial media and fruits. Similarly, Seal (8) distinguished at least two biologic forms of American *Sclerotinia*, as he calls it, which showed constant cultural differences when grown on various substrata and under different environ-

mental conditions. Roberts and Dunegan (7) also found cultural variations within strains on different media.

Sharvelle and Chen (9) observed that the 16 lines obtained from 2 asci of *S. fructicola* fell into 4 distinct groups on the basis of cultural characteristics, sensitivity to sulphur fungicides, and pathogenicity to apple fruits.

Ezekiel (3) reported the behavior of different strains when seeded in combinations of 2 per plate at different points in potato-dextrose-agar plates. He did not observe any macroscopic reaction where the 2 colonies grew together. On microscopic observation he noticed anastomosing of hyphae at the meeting line with some strains but not with others.

METHODS OF ISOLATING ASCOSPORES AND HANDLING STOCK CULTURES

The apothecial material employed was received from the States of Washington, Illinois, and Michigan in April–May, 1947. Eight ascospores from an ascus were isolated in the order of their linear arrangement in the ascus by the technique employed by Keitt and Langford (4). In addition, another procedure was followed with good success. Asci of suitable maturity, when placed on 4 per cent agar plates, discharged 8 ascospores together in a linear mass without, in a majority of cases, disturbing the order of their linear arrangement in the ascus. The 8 spores from an ascus were then separated, in the order of their linear arrangement in the ascus, with the use of a glass micro-needle. This procedure was followed only in cases in which the linear arrangement of the discharged spores was clearly maintained.

The 8 ascospores isolated from an ascus were arranged in serial order on a water-agar plate and their positions marked by making a scratch in the agar opposite each ascospore. The plate was then incubated at 20° C. for germination of the spores. When all the 8 spores had germinated, a cut in the agar was made around each. The agar blocks with germinated spores were placed on a potato-dextrose-agar (extract from 200 gm. potato, 20 gm. dextrose, 17 gm. agar, distilled water to make 1 liter) plate so that ascospore number 1 was in the center with the other 7 surrounding it in serial order. If one or more of the 8 spores failed to germinate, the whole ascosporic set was rejected.

On the second or third day when all the 8 monoascosporic lines were visible, triplicate transfers were made from each to potato-dextrose-agar slants which were incubated at the same temperature. Four-day-old cultures in these slants were covered with sterilized mineral oil and stored in a refrigerator at 4° C. for stock cultures. The latter were renewed every 4 months.

Complete ascosporic sets were isolated from 36 asci from the various apothecia as follows: 2 sets from 2 apothecia from Washington, 16 sets from 15 apothecia from Illinois, and 18 sets from 12 apothecia from Michigan.

The 8 ascospores from any ascus and the monoascosporic lines derived from them were given the serial numbers I ... VIII, according to the order of linear arrangement of the spores in the ascus, beginning at the apex. The 36 asci and the 36 ascosporic sets derived from them were given the serial numbers 1 ... 36.

BEHAVIOR OF ASCOSPORIC SETS IN COMBINATION

The 8 monoascosporic lines from each of the 36 asci were seeded in a potato-dextrose-agar plate as described above, incubated at 24° C., and examined every other day. After 4 to 6 days of incubation the 8 mono-ascosporic colonies in each case could be clearly grouped into 4 pairs on the basis of colony characters. Colonies of lines I and II, III and IV, V and VI, and VII and VIII, respectively, looked exactly alike and were designated as members of the first, second, third, and fourth pairs.

Along with the differentiation of monoascosporic colonies into pairs there began to appear a dark line wherever members of the different pairs were adjacent to each other, but never between the members of the same pair. In about 12 days of incubation these lines became darker and thicker. The thickness of the dark lines between different pairs was not always the same. In ascus 8 (Fig. 1, A) the lines between the first and fourth and the second and third pairs were much darker and thicker than those between the first and second, the first and third, and the third and fourth pairs. In some ascosporic sets double dark lines were formed between certain pairs and single dark lines between others.

The 8 monoascosporic lines of ascus 10 were selected to study further the formation of dark lines between these isolates. All 8 lines were seeded in each of 8 potato-dextrose-agar plates so that each line occupied the central position in one of the plates with the other 7 lines surrounding it. With this arrangement, each line was adjacent to each of the other 7 in a single plate, thus saving time and space. Each line was also seeded at 8 similar positions in a single plate to see if dark lines would appear between colonies arising from one monoascosporic line. Potato-dextrose-agar plates were seeded in 3 replicates for each of the above combinations and incubated for 14 days at 25° C. Dark lines appeared wherever colonies from any 2 pairs were adjacent to each other, but never between 2 monoascosporic isolates of the same pair. No dark line was observed between colonies derived from isolates of any one monoascosporic line. Similar results were obtained when 8 monoascosporic lines from each of the asci 1, 8, and 17 were seeded in the same way as those of ascus 10.

The 8 monoascosporic lines from each of the asci 8 and 10 were also seeded in all possible combinations of two in potato-dextrose-agar plates to study their interaction in more detail. The 2 lines were seeded at 2 opposite points towards the periphery of the plates, so that the colonies formed by them were adjacent to each other in the middle of the plate. The seeded

plates were incubated for 2 weeks at 24° C. It was observed that a dark line was formed wherever 2 monoascosporic lines from 2 different pairs were adjacent to each other. The dark lines varied from narrow, faint,

FIG 1 A Differentiation of the 8 monoascosporic isolates I VIII from ascus 8 into 4 pairs by colony characters and by dark lines formed between pairs on potato dextrose agar, after 12 days of incubation at 24° C B E The 4 monoascosporic isolates II, IV, V, and VIII of ascus 10 were seeded, respectively, in the potato dextrose agar plates. B E surrounded by the 4 biotypes from ascus 1 Each isolate of ascus 10 was delimited from all the isolates of ascus 1 by the formation of dark lines Dark lines were also formed between the 4 isolates of ascus 1 Photographed after 14 days of incubation at 24° C

and single to broad, very dark, and double. No dark line was formed when 2 monoascosporic lines from the same pair were adjacent to each other.

It is noteworthy that no two pairs, out of the 144 pairs isolated from 36 asci, were exactly alike as judged by macroscopic examination; only the members of a pair were alike in all cultural characteristics. This observation led to a study of the behavior of monoascosporic lines from different asci in various combinations. The 8 lines of ascus 10 were seeded singly in the center of potato-dextrose-agar plates and each was surrounded by the 8 monoascosporic lines of ascus 8. After 10 days of incubation at 24° C. each of the 8 lines of ascus 10 was delimited from all the surrounding 8 lines of ascus 8 by dark border lines. Like results were obtained when the monoascosporic lines of ascus 10 were seeded similarly with the lines from asci 1, 17, and 25, respectively. Another experiment was set up, in which each of the 4 monoascosporic lines from the 4 different pairs of ascus 10 was seeded in the center of a potato-dextrose-agar plate and each was surrounded by 4 lines from the 4 different pairs of asci 1 and 8, respectively. This procedure reduced the number of monoascosporic lines in one plate from 9 to 5 and was well suited for the purpose of taking photographs. The results obtained were the same as before (Fig. 1, B-E).

MORPHOLOGY OF DARK LINES

On microscopic observation it was found that the fungus produced abundant microconidia on the surface of dark lines. The mycelium in the dark line was brown, very fine and threadlike, and very much clustered and intertwined. The whole structure showed similarity to the sclerotial crust formed by the fungus in older cultures. Microconidia were produced on the dark lines as soon as the latter became visible, i.e., in about 8 days at 24° C.

Colonies of monoascosporic lines produced microconidia and formed sclerotial crust much later.

BEHAVIOR OF MONOASCOSPORIC LINES WHEN PLATED SINGLY AT DIFFERENT TEMPERATURES

It was found that the cultural details of the monoascosporic lines could be followed better when the lines were plated singly. All the 36 sets were thus plated in 3 replications on potato-dextrose agar at various temperatures. The behavior of cultures was examined every other day, and the details of colony characters of some representative types were recorded at the same intervals. In each set the 8 monoascosporic lines could be distinctly differentiated into 4 pairs on the basis of colony characters such as size, color, zonation, margin, amounts of mycelium and conidia produced, and pigmentation formed by the submerged mycelium. The colonies of the same pair looked exactly alike but differed from those of the other 3 pairs of the same set on the basis of the above cultural characters (Fig. 2, A, B,
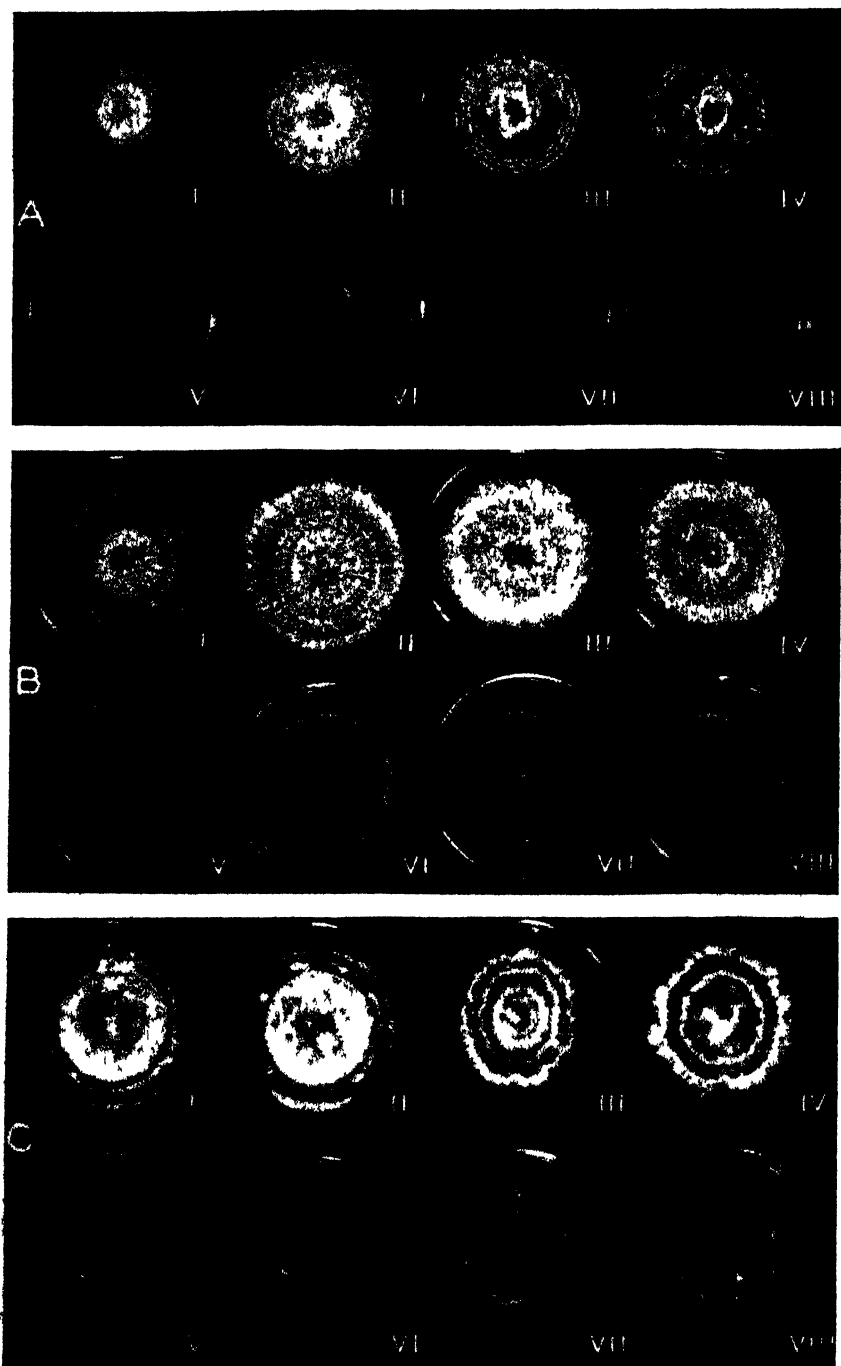
FIG. 2. Differentiation of the 8 monoascosporic isolates I-VIII from ascus 17 on potato-dextrose agar into 4 pairs on the basis of colony characters: A, after 19 days of incubation at 8° C.; B, after 12 days of incubation at 12° C.; C, after 10 days of incubation at 28° C.

and C). Likewise, colonies derived from a pair of any set differed from those derived from the rest of the 143 pairs of the 36 sets. The 4 pairs in most cases were clearly differentiated in 4 to 6 days at temperatures of 16°, 20°, 24°, 28°, and 32° C., in 6 to 8 days at 12° C., and in 12 to 18 days at 8° C.

RATE OF GROWTH AND TEMPERATURE RELATIONS

The 8 monoascosporic lines from each of the asci 1, 2, and 17 were plated singly in 3 replicates on nonacidified potato-dextrose agar (*p*H 5.6 to 5.8) and incubated at 4°, 8°, 12°, 16°, 20°, 24°, 28°, 32°, and 36° C., respectively. Mycelial disks of equal size, cut with the same metallic ring, were used for inoculum. Cultural characters and colony diameter were recorded every other day for 6 days. Growth at 4° C. was negligible or extremely slow. The 8 monoascosporic lines of ascus 17, left at 4° C. for 3 months, filled only one-quarter to one-half of the plates, but they could be clearly differentiated into 4 pairs. All lines from the 3 asci showed maximum growth at 24° C. Sectoring of most of the lines from the 3 asci was very frequent at 32° C., and the 8 lines from an ascus could not always be differentiated into 4 pairs at that temperature. None of the lines showed any growth at 36° C. The 8 lines from each of the 3 asci could be clearly differentiated into 4 pairs at 8°, 12°, 16°, 20°, 24°, and 28° C., although at certain temperatures the differentiation was more prominent than at others.

The rate of growth for the 8 lines from each of the 3 asci increased progressively from 8° to a maximum at 24° C., and then fell at higher temperatures. It would occupy too much space to record the rate of growth of the 8 lines from each of the 3 asci at all the temperatures studied. Therefore, the rate of growth of 8 lines from asci 1, 2, and 17, respectively, is represented graphically only at its maximum level at 24° C. (Fig. 3). The 8 lines in each of asci 1, 2, and 17 are represented in 4 pairs since the members of each pair showed about the same rate of growth. The 4 pairs from ascus 1 showed nearly the same rate of growth while those from asci 2 and 17 had quite different growth rates. The rate of growth of the 4 pairs from ascus 2 was in the order of fourth > first > second > third. It should be noted, however, that the first and the second pairs had nearly the same rate of growth. In ascus 17 the rate of growth of the 4 pairs was in the order of first > third > second > fourth. On the whole the rate of growth for the 3 asci was in the order of 17 > 1 > 2.

The above distinctions of the rate of growth of the pairs from the same ascus, as well as the averages per ascus for the 3 asci, held true, to a greater or less extent, at all the temperatures studied.

The data on the 6 days of growth of the 8 monoascosporic lines of each of the asci 1, 2, and 17, over the whole range of temperatures studied, are
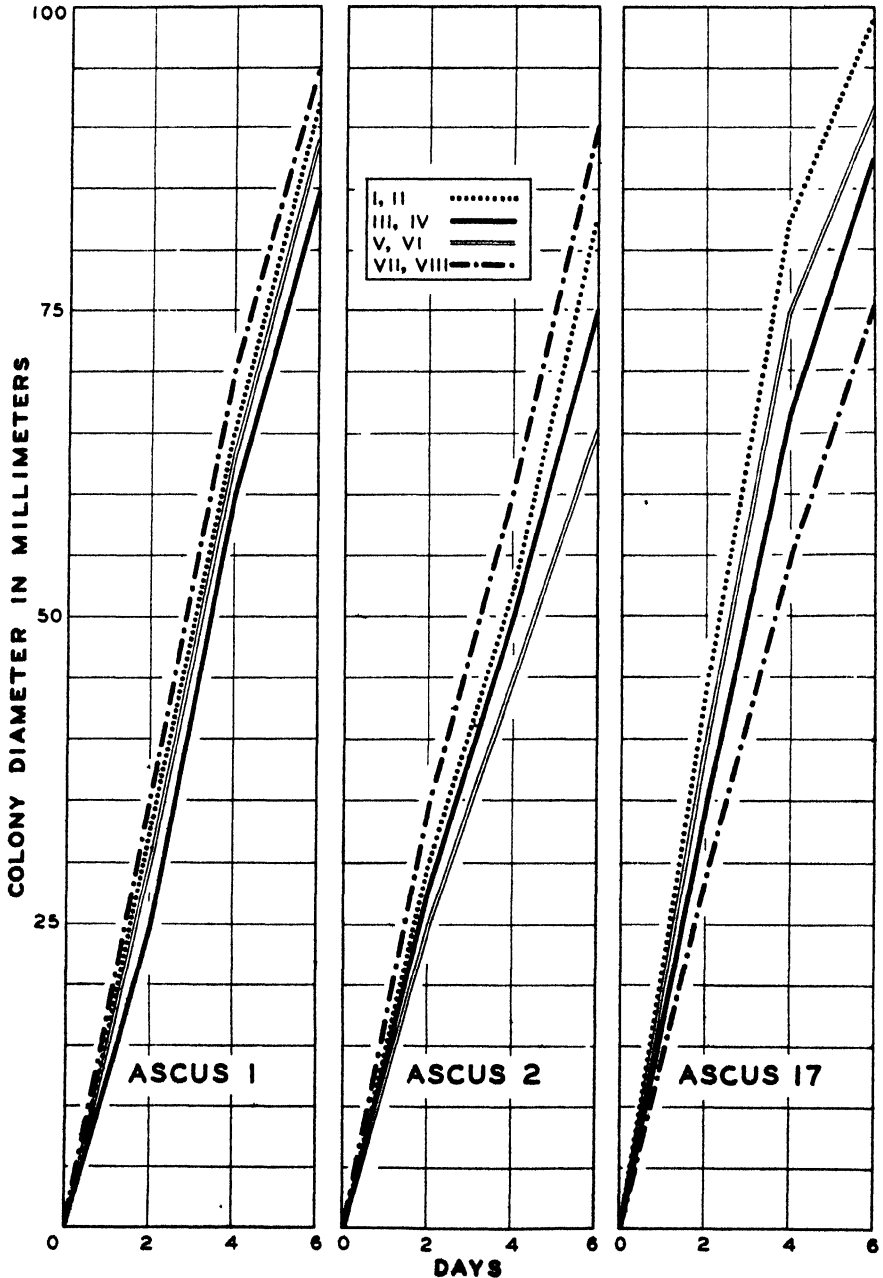
FIG. 3. Rate of growth of the 8 monoascosporic isolates I–VIII from asci 1, 2, and 17, incubated on potato-dextrose agar at 24° C. Growth was measured every other day for 6 days and the results from each pair of isolates were averaged.

summarized graphically in figure 4. The 8 lines of each of these asci are represented in 4 pairs since the members of each pair showed about the same amount of growth. As shown in the graphs, the growth was zero at

4° and 36°, while it increased progressively from 8° to 24° C., and then fell at 28° and 32° C. The distinctions as regards the amount of growth
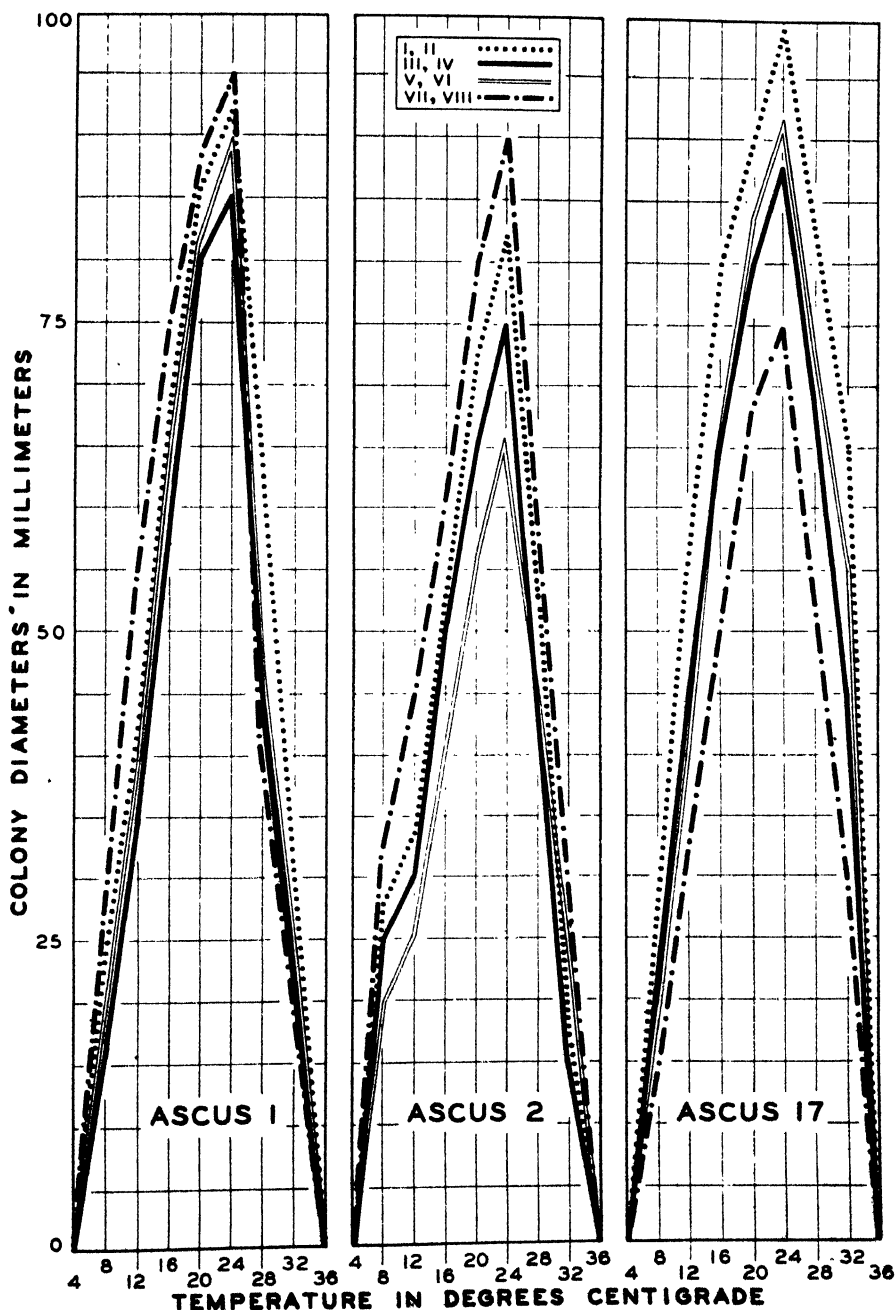
FIG. 4. Growth of the 8 monoascosporic isolates I-VIII from asci 1, 2, and 17 on potato-dextrose agar after 6 days of incubation at various temperatures. The results from each pair of isolates were averaged.

within pairs from the same ascus as well as among pairs from the 3 asci correspond closely to those concerning the rate of growth at each temperature and so need not be repeated here. The minima, optima, and maxima of temperatures for growth of all the lines from the 3 asci lie near 4°, 24°, and 36° C., respectively. This is in close agreement with the results obtained by earlier workers on various strains of *S. fructicola* (1, 3).

RELATIONS OF HYDROGEN-ION CONCENTRATION

The effect of hydrogen-ion concentration on the growth of the 8 monoascosporic lines of ascus 10 was determined on potato-dextrose agar adjusted, after autoclaving, to a pH range of 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 7.0, 7.5, 8.0, 8.5, 9.0, and 10.0. The pH ranges of 1.5 to 5.0 and 6.0 to 10.0 were adjusted with hydrochloric acid and potassium hydroxide, respectively. The above figures represent the hydrogen-ion concentrations checked after the potato-dextrose agar was solidified in poured plates. The 8 lines were seeded in 3 replicates with mycelial disks of equal size and incubated at 24° C. for 6 days. The cultural characteristics and the colony diameter were recorded every other day. At the end of 6 days of incubation the final pH was determined for each replicate with a Beckman pH meter. The whole colony along with the medium under it was crushed in a beaker with a small amount of distilled water and the pH determined. The data on the 6 days of growth in terms of colony diameter and the final pH obtained at each pH studied are summarized in table 1. The data on the 6 days of growth are also represented graphically in figure 5. The 8 monoascosporic lines are represented in 4 pairs in figure 5, since members of each pair had almost the same amount of growth, as shown in table 1. There was no growth at pH 1.5 and 10.0, while it was very slight at pH 9.0. The growth of all the monoascosporic lines increased progressively from pH 2.0 to 4.0, leveled off up to pH 5.0, and then fell at higher hydrogen-ion concentrations. The slope of the curves is very steep from pH 2.0 to 4.0 and again from pH 7.0 to 9.0, while it is rather gradual from pH 5.0 to 7.0. The growth of all monoascosporic lines was maximum in the pH range of 4.0 to 5.0.

The data on final pH in table 1 indicate that pH always shifts to the acid side for all initial hydrogen-ion concentrations except 2.0, 2.5, and 3.0, which did not change after 6 days' growth. This shows that the growth of all monoascosporic lines makes the medium more acidic.

The 8 monoascosporic lines could be clearly differentiated into 4 pairs over the whole range of pH from 2.0 to 9.0 on the basis of amount of growth and pH changes induced. Members of each pair gave almost the same amount of growth and the same final pH but differed from those of the other 3 pairs in those respects. The order of the amount of growth and the changes in pH produced by the 4 pairs was not the same, however, at all hydrogen-ion concentrations. The order of the amount of growth of the 4 pairs depended upon the pH used.

TABLE 1.—*Effect of initial pH of potato-dextrose-agar medium on the growth of the 8 monoascosporic isolates from ascus 10 of S. fructicola*

Initial pH	\	\	\	\	Data from stated monoascosporic lines:											
	I		II		III		IV		V		VI		VII		VIII	
	C.D.[a]	pH[b]	C.D.	pH	C.D.	pH	C.D.	pH	C.D.	pH	C.D.	pH	C.D.	pH	C.D.	pH
1.5	0	1.5	0	1.5	0	1.5	0	1.5	0	1.5	0	1.5	0	1.5	0	1.5
2.0	20	2.0	21	2.0	18	2.0	19	2.0	22	2.0	23	2.0	19	2.0	20	2.0
2.5	25	2.5	26	2.5	21	2.5	21	2.5	38	2.5	37	2.5	27	2.6	28	2.5
3.0	42	3.0	43	3.0	36	3.0	37	3.0	50	2.9	49	2.9	45	2.9	46	2.9
4.0	85	3.8	85	3.8	75	3.9	75	3.9	93	3.8	90	3.7	70	3.6	65	3.7
5.0	85	4.0	85	4.0	75	4.0	75	3.8	90	3.8	93	3.8	65	3.8	70	3.9
6.0	75	4.3	75	4.3	65	4.4	65	4.4	80	4.1	85	3.8	60	4.1	65	4.1
7.0	70	4.5	70	4.5	58	4.7	60	4.7	80	4.3	80	4.1	55	4.2	50	4.2
7.5	50	4.6	50	4.6	40	4.7	40	4.7	60	5.2	65	4.3	40	4.4	45	4.4
8.0	30	5.4	35	5.4	35	4.9	40	4.9	25	6.9	24	5.2	35	4.9	40	4.8
8.5	17	6.6	18	7.0	22	7.1	23	7.0	12	8.6	13	7.0	25	5.5	26	5.4
9.0	5	7.8	5	8.0	6	8.2	6	8.3	5	9.8	5	8.5	7	8.6	7	8.6
10.0	0	9.8	0	9.8	0	9.7	0	9.7	0		0	9.7	0	9.7	0	9.8

[a] Colony diameter in millimeters after 6 days of incubation at 24° C.
[b] Final pH.

Apart from the amounts of growth produced, the 8 lines of ascus 10 could be clearly differentiated into 4 pairs at the whole range of pH 2.0 to 9.0 on the basis of other colony characters.

DISCUSSION

Roberts and Dunegan (7) reported that the monostichous arrangement of the ascospores in the asci of *S. fructicola* becomes irregular just before the spores are discharged. However, the observations made in the present studies indicate that while this is true of some asci it is not a rule for all of them. In the majority of the cases observed the monostichous arrangement of ascospores was retained not only just before their discharge but even after they were actually discharged. Advantage was taken of this phenomenon to devise a method to isolate ascospores in the order of their linear arrangement in the ascus after they are discharged. The method is convenient and well adapted for the asci of *S. fructicola*.

The 8 monoascosporic lines from each of the 36 asci studied were differentiated into 4 distinct pairs on the basis of colony characters. Furthermore, it was shown, on the basis of the same characters, that each pair is differentiated not only from the other 3 pairs of the same ascus but also from all the 140 pairs of the remaining 35 asci. The cultural differences between pairs were maintained throughout the present studies.

All the 36 monoascosporic sets gave 4 pairs in the same sequence, *i.e.*, ascosporic lines I and II formed the first, III and IV the second, V and VI the third, and VII and VIII the fourth pair. In this respect *S. fructicola* seems unusually well adapted for certain types of genetic work.

Sharvelle and Chen (9) reported that the 16 lines obtained from 2 asci yielded 4 distinct groups on the basis of cultural characteristics, sensitivity to sulphur fungicides, and pathogenicity on apple fruits. However, they did not report isolating the ascospores in the order of their linear arrangement in the ascus, which is considered a desirable procedure in order to follow the comparative cultural details of biotypes.

The 8 monoascosporic lines from each of the 36 asci were further differentiated into 4 distinct pairs on the basis of the dark lines, which appeared only between pairs but never between members of the same pair. It was demonstrated with reference to asci 1, 8, 10, and 17 that dark lines were formed between any pairs of the same ascus. A dark line was also formed wherever any pair from ascus 10 was adjacent to any pair from any of the asci 1, 8, 17, and 25. There was a great variation in the pattern of dark lines between different pairs, indicating that pairs differed from one another as regards this response.

The mycelium of the dark lines looks sclerotial in character and produces abundant microconidia. It should be remarked here that the formation of sclerotial bodies precedes the development of apothecia in *S. fructicola* and other species of the genus. Moreover, Drayton (2) has demon-
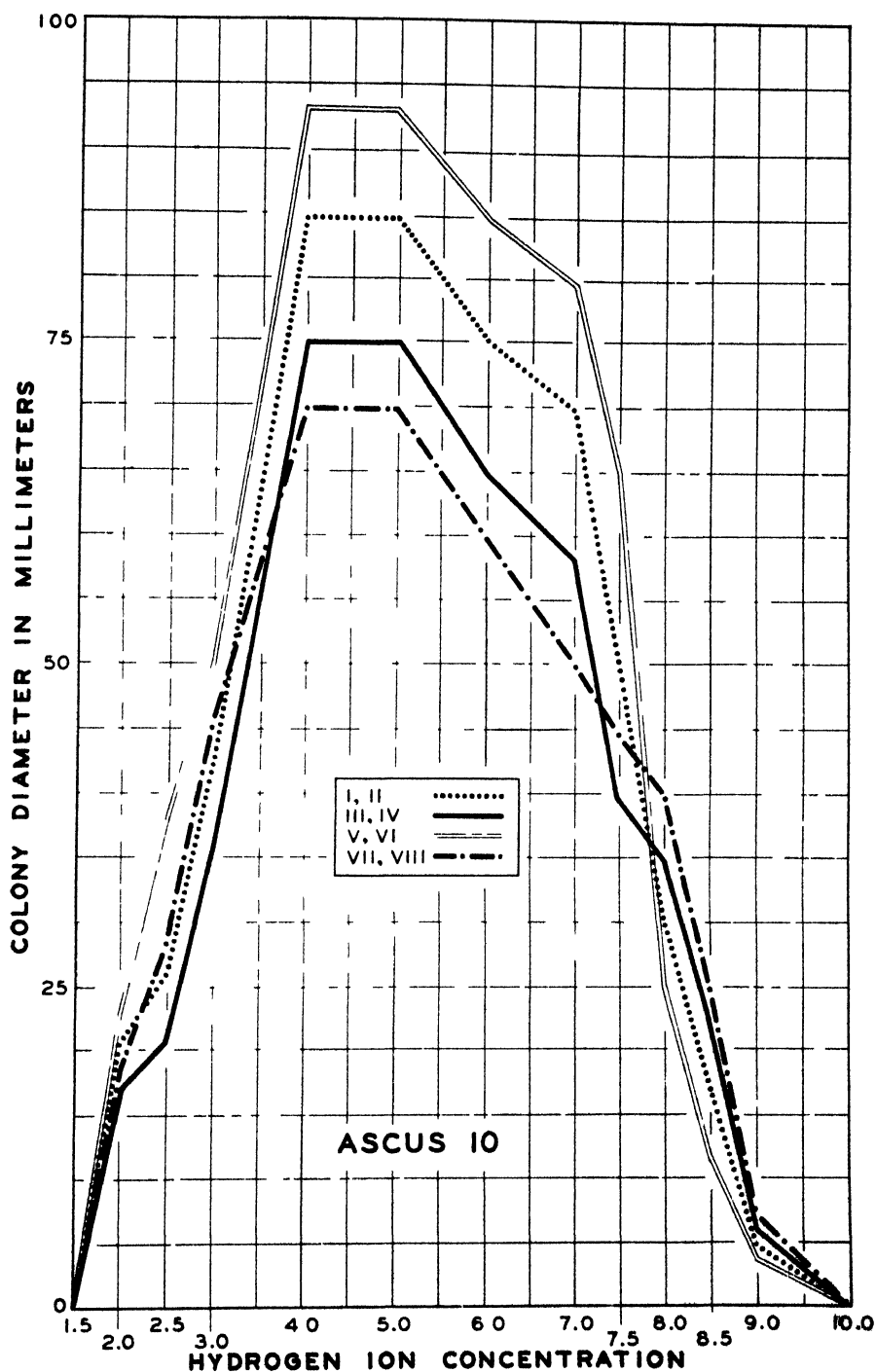
FIG. 5. Growth of the 8 monoascosporic isolates I-VIII from ascus 10 on potato-dextrose agar which was adjusted to various initial hydrogen-ion concentrations. Results were taken after 6 days of incubation at 24° C. and averaged for each pair.

strated in the case of *S. gladioli* that the microconidia are functional male
organs.

Ezekiel (3) seeded different strains of the Sclerotinias he worked with
in pairs on potato-dextrose-agar plates and looked for evidences of reaction
between strains at the junction of the colonies. He did not report occur-
rence of dark lines. It is possible that the cultural conditions he employed
did not permit the development of the dark lines, or that he may have used
isolates that would not react to give them. While some of the pairing was
between different species, at least one pair seems to have been between
strains of *S. fructicola*.

Formation of dark lines between different strains has been described by
different workers on various fungi and the phenomenon has been termed
by many as aversion or inhibition. A brief review of the literature on
aversion is given by Voorhees (11). Various workers have interpreted
aversion as a result of physiological, chemical, or sexual response when
2 different strains come into contact with each other. It is possible that the
formation of the dark lines encountered in the present studies has some
connection with a sexual cycle. However, the elucidation of this phenome-
non must await the results of further investigation.

The 8 monoascosporic lines of each ascus studied were clearly differen-
tiated into 4 pairs on the basis of colony characters at all temperatures and
hydrogen-ion concentrations that permitted their growth. However, at cer-
tain temperatures and hydrogen-ion concentrations the differences between
pairs were more marked than at others. Mader and Teller (5) reported
that culturing *Sclerotinia* spp. on potato-dextrose agar at pH 3.1 to 3.7
facilitated differentiation of cultural types. They also state that 5 asco-
spores from a single ascus yielded 3 cultural types. They do not give the
names of the species dealt with.

Evidence from colony characters, formation of dark lines, and hydrogen-
ion and temperature relations is in agreement in showing that each ascus
studied contained 4 genetically different biotypes. It is therefore con-
cluded that segregation of genetic factors regularly occurs in the asci of
S. fructicola and that genetic combinations and segregations incident to
development of the apothecial stage are a major factor in heritable varia-
tion in this pathogen.

Attempts of the writers to produce the ascigerous stage of the fungus
experimentally have thus far failed to yield positive results, but the time
and opportunities for experimentation with monoascosporic isolates have
been very limited. If satisfactory methods can be developed for breeding
this pathogen experimentally, it would afford promising material for basic
studies.

SUMMARY

Apothecial material of *Sclerotinia fructicola* (Wint.) Rehm was re-
ceived from the States of Washington, Illinois, and Michigan and studies
were made on the variability of numerous monoascosporic lines.

The 8 ascospores were isolated in the order of their linear arrangement in the ascus from each of 36 asci from 29 apothecia.

The 8 monoascosporic lines from each of the 36 asci, when plated singly on potato-dextrose agar at 24° C., were differentiated into 4 distinct pairs on the basis of colony characters. Each pair was thus differentiated not only from the other 3 pairs of the same ascus but also from the 4 pairs derived from each of the other 35 asci.

The 4 pairs from each of the asci 1, 2, and 17 were similarly differentiated clearly at all temperatures studied that permitted their growth (8° to 32° C.). A temperature of 24° C. was found to be optimum for the 4 pairs of each of these asci.

The 4 pairs from ascus 10 were also differentiated clearly on the basis of colony characters at all hydrogen-ion concentrations studied that permitted growth (pH 2.0 to 9.0). A pH range of 4.0 to 5.0 was found to be optimum for the growth of these 4 pairs.

The 8 monoascosporic isolates from each of the 36 asci when cultured in suitable arrangement in the same potato-dextrose-agar plate were also differentiated into 4 distinct pairs on the basis of the appearance of dark lines between pairs.

It was demonstrated with reference to asci 1, 8, 10, and 17 that dark lines were formed between any 2 pairs of the same ascus.

A dark line was also formed whenever any pair from ascus 10 was adjacent to any pair from any of the asci 1, 8, 17, and 25.

No dark line was ever found between the 2 members of one pair.

Between some pairs there were formed double dark lines instead of a single one. Dark lines also varied from faint and narrow to very dark and very thick.

The mycelium of the dark lines seems to be sclerotial in character and produces abundant microconidia.

It is concluded that each monoascosporic pair was genetically different from all the other monoascosporic pairs whether derived from the same ascus or from the other asci studied. It is apparent, then, that segregation of genetic factors occurs in the asci of *S. fructicola* and that each ascus contains four biotypes.

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PHYTOPATHOLOGY [VOL. 39

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RECOVERY OF PLANTS FROM DODDER LATENT MOSAIC

C . W . B E N N E T T

INTRODUCTION

Complete or nearly complete recovery from primary symptoms of virus disease in plants has been reported in several instances, notably with ring spot of tobacco (*Nicotiana tabacum* L.) (7), curly top of tobacco (2, 5, 6) and curly top of tomato (*Lycopersicon esculentum* Mill.) (3, 6). It has been reported more recently that most species and varieties of plants that show marked symptoms of dodder latent mosaic following infection, later completely recover from symptoms or show marked improvement (1).

Dodder latent mosaic appears to possess certain characteristics of special value for studies of the recovery phenomenon. Among these characteristics may be noted (a) a rather wide host range permitting studies on plants widely separated botanically; (b) host plants such as pokeweed (*Phytolacca americana* L.) and celery (*Apium graveolens* L.) that pass through a series of changes in the process of recovery involving necrosis or intense chlorosis followed by leaf spotting or mottling; and (c) ability of the causal virus to produce local lesions on pokeweed, thus providing a means by which relative virus concentrations may be estimated with greater ease and accuracy.

This paper reports results of further studies on recovery of plants from symptoms of dodder latent mosaic and on some of the changes in virus concentration associated with injury and recovery.

MATERIALS AND METHODS

The species of plants used in studies of recovery from dodder latent mosaic include sugar beet (*Beta vulgaris* L.), pokeweed, cantaloupe (*Cucumis melo* L., var. Rocky Ford), celery, tomato, and potato (*Solanum tuberosum* L.), all of which show marked symptoms at first. Certain other species were used, on which only mild symptoms or none at all were observed, but which are known to carry the virus.

Tests of virus concentration were made by the primary lesion method on pokeweed. Test plants were first sprinkled with an abrasive and then inoculated by passing a cloth pad saturated with inoculum lightly over the surface of each leaf three times after which the leaves were washed with a light spray of water. All of the mature leaves of each plant were inoculated and the lesions on 3 leaves, usually but not always consecutive, showing the greatest number of lesions were counted on each plant. In making determinations of comparative virus concentrations of two virus samples the half-leaf method was used and four plants were inoculated with each sample in

each test. The inoculations were made in such a manner as to give equal representation to each of the two samples with respect to relative time of inoculation into the leaf and the side of the leaf into which the inoculum was introduced.

GENERAL PATTERN OF RECOVERY

The pattern of recovery, in general, is more or less similar with all of the species of plants studied, but certain minor variations are evident. On sugar beet, first symptoms usually consist of circular yellow spots sometimes so closely spaced as to cause yellowing of much of the leaf surface. Spotting and yellowing are accompanied by a certain amount of distortion and dwarfing. As the plants grow, symptoms become progressively milder on the younger leaves and after the production of four to eight leaves with symptoms the new growth is normal in appearance.

Severe necrosis is an early symptom on rapidly growing pokeweed plants. This usually appears first on the base of one leaf, and the next younger leaf may be killed. Necrosis appears on two or three other leaves, becoming progressively less severe until it grades into mottling which appears on three or more leaves. Later growth is normal or nearly so.

On Golden Self Blanching celery, usually a half-grown leaf begins to show chlorosis extending outward into the leaflets from the midrib. The next leaf may be almost completely chlorotic and then the plant passes into a condition in which the chlorosis grades into mottling in two or three leaves that are much stunted and crinkled. Leaves produced later are normal in size and in general appearance.

On Rocky Ford cantaloupe early symptoms consist of necrotic and chlorotic spots along the veins. Often the affected leaves are crinkled and spotted and of less than half normal size. After the production of three to five such leaves the plant begins to recover and passes into a chronic stage in which the leaves are smaller than those on noninfected plants and have chlorotic spots about 2 mm. in diameter that may become necrotic in the centers as the leaf becomes older.

RECOVERED PLANTS UNAFFECTED BY REINOCULATION

Plants recovered from the effects of dodder latent mosaic virus do not produce additional symptoms following a second inoculation. This has been shown in tests of sugar beet, Golden Self Blanching celery, and pokeweed, in which recovered plants were inoculated by means of *Cuscuta californica* Choisy and *C. campestris* Yuncker. All recovered plants inoculated in this manner remained free of symptoms.

Tests were made also with the primary lesion technique on pokeweed. Leaves on recovered pokeweed plants failed to produce primary lesions following juice inoculation by the rubbing method; whereas leaves of the same size and age on healthy plants produced many lesions on each inoculated leaf.

RELATIVE CONCENTRATION OF VIRUS IN RECOVERED AND
RECENTLY INFECTED PLANTS

Sugar beet, celery, pokeweed, tomato, cantaloupe, and potato were selected for comparison of virus concentration in recently infected and recovered individuals. With the exception of cantaloupe, all of these plants almost completely recover from symptoms. Plants of each kind were inoculated and retained until new growth with a maximum degree of recovery was produced. Young leaves or shoots from these recovered plants were selected and their virus content compared with that in severely affected leaves or shoots of plants of the same age but more recently infected.

The results of three series of tests of each of these species of plants are shown in table 1. Without exception virus concentrations found in recov-

TABLE 1.—*Relative concentrations of dodder latent mosaic virus in recovered and recently infected plants of the same species, as indicated by local lesion counts on pokeweed*

Plant tested	Average number of lesions per half leaf produced with juice from recovered plants in test No.			Average number of lesions per half leaf produced with juice from recently infected plants in test No.		
	1	2	3	1	2	3
Sugar beet	2.3	1.1	1.8	26.7	27.0	16.0
Celery	0.1	2.2	0.3	37.2	97.3	34.9
Pokeweed	1.7	1.2	1.4	30.9	34.6	50.5
Tomato	0.1	0.7	1.0	27.1	72.2	73.5
Cantaloupe[a]	0.3	4.0	0.6	92.7	18.2	25.4
Potato	2.6	3.2	2.7	31.2	16.1	26.5

[a] Tests were made with plants showing chronic symptoms and with recently infected plants showing necrosis and leaf crinkling.

ered plants were very low compared with those in the diseased portions of more recently infected plants showing severe symptoms.

CONCENTRATION OF VIRUS IN SYMPTOMLESS CARRIERS

Certain plants, particularly Turkish tobacco, *Nicotiana rustica* L., *N. glauca* R. Grah., *N. glutinosa* L., *Brassica adpressa* (Moench) Boiss., and *Cuscuta californica*, have been found to carry the virus of dodder latent mosaic over long periods but no symptoms of disease have been observed on any of these plants except on some plants of *Nicotiana glutinosa* on which a type of vein clearing occurred.

Several tests were made to determine relative concentrations of virus in plants of each of these species. The results of three representative tests in which virus concentration was compared with that in recently infected pokeweed are shown in table 2.

These results indicate that the concentrations of virus in plants of these species were very low, even somewhat lower than those in the recovered

plants shown in table 1. In fact, no virus was demonstrated to be present in certain plants of some of the species by the local-lesion technique. However, in all cases virus was transmissible readily from such plants to pokeweed by means of *Cuscuta californica*.

TABLE 2.—*Relative concentrations of virus in recently infected pokeweed plants and in plants of species that are symptomless carriers, as indicated by local lesion counts on pokeweed*

Symptomless carrier tested	Average number of lesions per halfleaf on plants inoculated with juice from indicated symptomless carrier in test No.			Average number of lesions per halfleaf on plants inoculated with juice from recently infected pokeweed in test No.		
	1	2	3	1	2	3
Nicotiana glauca[a]	0.0	0.0	0.0	54.1	12.1	15.8
Nicotiana tabacum, var. Turkish	0.0	0.1	1.0	11.1	63.5	18.1
Nicotiana glutinosa[b]	1.0	0.3	0.0	29.7	56.2	14.9
Nicotiana rustica	0.0	0.2	0.1	17.5	28.7	21.0
Brassica adpressa	0.5	0.1	0.2	38.1	22.2	11.0
Cuscuta californica	0.4	1.4	0.0	21.8	50.5	45.8

[a] Virus was later transmitted from these plants to pokeweed by means of *Cuscuta californica*.

[b] Some infected plants of this species showed vein chlorosis, others did not. Plants showing no symptoms were used in these tests.

Plants used in these tests had been infected more than three weeks and, therefore, the concentrations of virus determined were those reached after periods long enough to permit recovery in plants of more susceptible species. Since plants of these species have been infected only by means of dodder and appear to be immune from or very resistant to infection by juice inoculation, the exact time of entrance of the virus into inoculated plants is difficult to determine. This interferes somewhat with the studies of fluctuation

TABLE 3,—*Fluctuations in virus concentrations in Cuscuta campestris following inoculation of pokeweed plants on which it was growing*

Test No.	Average number of local lesions per half leaf produced by juice from dodder the indicated number of days after inoculation of the pokeweed plants on which the dodder was growing														
	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34
1	0	0	5	14	76	21	10	13	12	3	3	3	0	0	4
2	0	7	6	4	28	25	4	4	3	3	2	4	3	1	2
3	0	3	10	12	19	48	21	5	3	2	4	3	2	3	1
4	0	1	8	17	29	54	39	12	4	2	2	3	1	4	2

of virus concentration in inoculated plants after infection. However, *Cuscuta campestris* is a host of the virus and since it grows well on pokeweed, a plant which is susceptible to infection by juice inoculation, it was utilized for a study of fluctuations in virus concentration in a symptomless carrier.

In making these tests seedling plants of *Cuscuta campestris* were established on small plants of pokeweed. After the pokeweed plants had reached a height of 6 to 8 in. and the dodder was growing vigorously they were inoculated with dodder latent mosaic virus by rubbing inoculum over the surfaces of the leaves. Systemic infection began to appear on the pokeweed plants usually in 8 to 10 days. Beginning with the sixth day after inoculation, dodder was removed from the inoculated plants and tested at intervals of 2 days over a period of 28 days for relative amount of virus present in the expressed sap as indicated by local lesions produced by juice inoculated into pokeweed.

The results of four tests, shown in table 3, indicate rather clearly that virus concentration increased rapidly in *Cuscuta campestris* following infection. The concentration remained at a high level a relatively short time and then declined to a lower level at which it appeared to be maintained. These fluctuations probably follow more or less closely those that occur in the pokeweed plants on which the dodder was growing. It does not seem likely, however, that virus concentration in dodder would be greatly influenced by that in the pokeweed. Evidence indicates that in all cases where high concentrations of any virus of the mosaic-producing type occurs in dodder it is due to multiplication in dodder rather than to movement from the host plant into dodder. It seems probable, therefore, that the concentrations of virus present in dodder in this case were largely those produced by multiplication in the dodder. Since no symptoms were observed in dodder plants that gave evidence of containing virus concentration of the same order as those in plants of other species that showed symptoms and then recovered, it seems probable that *Cuscuta campestris*, and possibly other symptomless carriers, have greater tolerance of virus than do plants such as pokeweed in which symptoms are associated with high virus concentrations.

INCREASE OF VIRUS OF DODDER LATENT MOSAIC IN THE PRESENCE OF THE
VIRUSES OF TOBACCO ETCH AND TOBACCO MOSAIC

After preliminary tests indicated that the addition of the virus of tobacco etch to tomato plants (var. Riverside) infected with dodder latent mosaic resulted in considerable additional stunting, studies were made to determine the effect of combinations of the two viruses on the plant and on concentrations of virus present. In each of a series of tests, 10 plants were inoculated with dodder latent mosaic virus, 10 with dodder latent mosaic virus plus tobacco etch virus, and 10 with tobacco etch virus. All inoculations were made by means of *Cuscuta californica* when the plants were about 10 cm. tall.

The plants inoculated with dodder latent mosaic virus usually became diseased after 8 to 12 days. Necrotic spots were produced on 2 to 5 young leaves and then the plants recovered and produced no symptoms on subsequent growth (Fig. 1, A). Plants inoculated with tobacco etch virus showed

symptoms usually in about 10 to 14 days.   Young leaves had a coarse pattern of mottling and leaves were slightly cupped and curled or twisted but not reduced in size (Fig. 1, C).   Plants inoculated with both viruses usually

FIG. 1.   Tomato plants inoculated with viruses causing dodder latent mosaic and tobacco etch: A, dodder latent mosaic virus alone; B, both dodder latent mosaic virus and tobacco etch virus; C, tobacco etch virus alone.   All inoculations were made at the same time and when the plants were about 10 cm. tall.   Plants A and C were about 90 cm. tall when photographed.

produced 4 to 6 leaves severely affected by necrosis.   Subsequent growth was much dwarfed, leaves were mottled, curled and twisted, and usually had varying numbers of small necrotic spots.   Plants grew to about half normal size (Fig. 1, B).

In a second series of tests, plants completely recovered from symptoms of dodder latent mosaic were inoculated with the virus of tobacco etch and plants with well defined symptoms of tobacco etch were inoculated with the virus of dodder latent mosaic.   In both cases the plants developed marked

symptoms of dodder latent mosaic after the second inoculation. Necrotic spots were produced on young leaves, some leaflets died, and the leaves became more conspicuously mottled, curled, and dwarfed. As the plants continued to grow necrosis became less evident, usually being limited to small inconspicuous spots, but the plants were dwarfed to about half normal size. Thus the severe effects due to the combination of viruses were produced regardless of which virus was introduced first.

Tests similar to those already described with the viruses of dodder latent mosaic and tobacco etch were made in which the virus of tobacco mosaic was substituted for tobacco etch virus. Inoculations with tobacco mosaic were made with juice from infected tobacco plants and those with dodder latent mosaic were made by means of *Cuscuta californica*. Results on tomato plants infected with both viruses were similar to those on plants infected with dodder latent mosaic and tobacco etch viruses except that leaves of plants containing tobacco mosaic virus showed more conspicuous mottling and a greater amount of deformity. As with the virus of tobacco etch, the introduction of the virus of tobacco mosaic into plants recovered from dodder latent mosaic resulted in necrosis followed by a certain degree of recovery but later growth was characterized by stunting, mottling, and deformities.

After the infected tomato plants had made considerable growth following infection, tests were made to determine relative concentration of dodder latent mosaic virus in plants infected by this virus alone compared with plants infected by both dodder latent mosaic virus and tobacco etch virus. Table 4 shows the results obtained from 5 pairs of plants of one experiment selected as representative of results of all tests. As indicated, the concentration of dodder latent mosaic virus appeared to be very low in all plants infected by this virus alone; whereas, the concentration in plants infected by both viruses was much higher.

Tests were made also to determine the relative concentration of the virus of tobacco etch in plants infected by both viruses as compared with plants with tobacco etch alone. In these tests juice was taken from the two sources, diluted 1–100, 1–500, 1–1000, and 1–2000. Ten healthy Turkish tobacco plants were inoculated with each dilution in each test. Five pairs of infected tomato plants were tested. Comparison of relative amount of infection obtained from the two virus sources did not indicate that the concentration of the tobacco etch virus was influenced by the presence of dodder latent mosaic virus.

Other tests were made to determine relative concentrations of dodder latent mosaic virus in plants infected by this virus alone and plants infected by dodder latent mosaic virus in combination with the virus of tobacco mosaic. Results of tests selected as representative are presented in table 4. These results show that the presence of the virus of tobacco mosaic in plants infected by dodder latent mosaic virus also resulted in a relatively large increase in the concentration level of the latter virus.

PHYTOPATHOLOGY

Tests were made also to determine whether the presence of the dodder latent mosaic virus in combination with tobacco mosaic virus resulted in increase or decrease of the latter virus in the infected plants. Juice from plants with tobacco mosaic alone and juice from plants with both viruses

TABLE 4.—*Comparison of concentration of the virus of dodder latent mosaic in tomato plants with dodder latent mosaic alone, dodder latent mosaic plus tobacco etch, and dodder latent mosaic plus tobacco mosaic, by the local lesion method on pokeweed*

Test number	Average number of lesions produced per half leaf by dodder latent mosaic virus in juice from tomato plants infected with			
	Dodder latent mosaic alone	Dodder latent mosaic plus tobacco etch	Dodder latent mosaic alone	Dodder latent mosaic plus tobacco mosaic
1	4.0	41.3	1.0	28.5
2	6.6	38.9	1.2	20.9
3	1.8	23.3	0.3	14.8
4	1 1	21.4	0.8	23.8
5	2.1	37.3	0.5	22.7

was diluted 1–100, 1–1,000, 1–10,000, and 1–100,000 and the concentration of virus in the respective dilutions of juice from the two sources compared by the half-leaf method on *Nicotiana glutinosa*. Results of these tests indicated that the presence of dodder latent mosaic virus in the plant had no influence on the concentration of the virus of tobacco mosaic.

DISCUSSION

It is evident that in the species and varieties of plants studied, recovery from dodder latent mosaic is associated with a marked reduction in concentration of the causal virus in the recovered plant. This reduction appears to be even greater than that found by Price (4) in plants recovered from tobacco ring spot. Associated with the mechanism of recovery in this case, therefore, is ability on the part of the plant to reduce appreciably or restrict virus increase following an initial period of rapid increase. Whether virus concentration falls to a level at which it is incapable of producing serious injury or whether recovered plants actually acquire a resistance to disease by virtue of the production of antibodies or other protective substances is a question of considerable importance but one which has not yet been answered fully.

Some of the results obtained with double infection on tomato appear to have a bearing on this point. When tomato plants, recovered from the effects of dodder latent mosaic, were infected with the virus of tobacco etch or the virus of tobacco mosaic, the concentration of dodder latent mosaic virus increased and became established at new levels considerably higher than those in tomato plants with dodder latent mosaic alone. It is evident, therefore, that the introduction of the second virus caused a modification of the mechanism controlling concentration of dodder latent mosaic virus. It is significant that this modification and the subsequent increase in con-

centration of dodder latent mosaic virus resulted in the reappearance of dodder latent mosaic symptoms in recovered plants and that marked symptoms continued to be produced as long as the plants were retained. It seems clear from this result that the introduction of the second virus into the recovered tomato plant destroyed the ability of the recovered plant to continue to protect itself against dodder latent mosaic. If the original resistance which the plant possessed resulted from the presence of antibodies or other protective substances, it is evident that after the introduction of the second virus these protective substances were suppressed or destroyed, or for some other reason failed to protect. It seems more probable that recovery was due to other factors and that the recovered plants did not acquire resistance or "immunity" to virus action but escaped injury owing to the fact that only virus concentrations too low to produce injury were present in the recovered plants. This supports the view that the critical factor in recovery and "immunity" of this type is control of virus increase. It may be that "immunized" plants are no more resistant to virus action than noninoculated plants and that any condition that results in a further virus increase in an "immunized" plant may result in the production of injury.

SUMMARY

Several species and varieties of plants including sugar beet, celery, pokeweed, tomato, and potato, show marked symptoms of disease after infection with dodder latent mosaic virus but completely or almost completely recover from symptoms as the affected plants continue to grow. Several other species and varieties, including *Nicotiana glauca, N. rustica, N. tabacum* var. Turkish, *Brassica adpressa,* and *Cuscuta* spp. are hosts of the virus but show no recognizable symptoms of disease.

Recovery was accompanied by marked reduction in virus concentration in the portions of the plant showing this recovery. Virus concentrations in symptomless carriers infected for more than 3 weeks were very low compared with those in recently infected pokeweed plants. It was found, however, that virus concentration reached a relatively high level in the symptomless carrier *Cuscuta campestris* a few days after infection and then receded to a relatively low level.

When recovered tomato plants were inoculated with tobacco etch or tobacco mosaic virus, symptoms of dodder latent mosaic again appeared on the young leaves and the plants remained dwarfed and continued to show symptoms of dodder latent mosaic. The dwarfed condition resulted regardless of the sequence of introduction of the virus components of the mixture. The concentration of the virus of dodder latent mosaic in the dwarfed plants appeared to be established and maintained at new levels much higher than those in recovered plants affected by dodder latent mosaic alone. The presence of dodder latent mosaic virus, however, appeared to have no influence on the concentrations of tobacco etch or tobacco mosaic virus.

The critical factor in recovery of plants from dodder latent mosaic appears to be restriction of virus to levels of concentration too low to produce marked symptoms of disease; possibly any condition that results in increased virus concentration may result in increased injury.

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SEPTORIA BLIGHT RESISTANCE IN THE TOMATO[1]

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INTRODUCTION

This paper reports the principal results obtained in attempts to develop commercially acceptable tomatoes possessing satisfactory resistance to defoliation caused by *Septoria lycopersici* Speg. In most years in the tomato canning acreages of the central states, Septoria leaf spot is the disease responsible for most severe defoliation, and in epidemic years it may cause a loss of 80 per cent of the foliage by early September. This degree of defoliation not only greatly reduces the photosynthetic activity of the plants but results in a significant decrease in quality due to sun scalding. Attempts to control the disease by the application of fungicides have not been impressive enough to be adopted by most commercial growers. Consequently, the development of resistant varieties would be desirable.

The varieties Essary and Break O'Day were originally reported (4, 11) as possessing considerable resistance to Septoria leaf spot. Levin (6), Pritchard and Porte (10), Alexander *et al.* (2), Locke (8), and Andrus and Reynard (3) have demonstrated, however, that commercial varieties of tomatoes possess no significant degree of resistance. Certain species of *Lycopersicon* have been shown to possess resistance. Thus Wright (15) reported a highly resistant selection of *L. peruvianum* (L.) Mill. var. *humifusum* C. H. Mull., and Wright and Lincoln (16) reported that F_1 plants of a cross of Rutgers with a selection of *L. hirsutum* Humb & Bonpl. P.I. 126445 were highly resistant to Septoria leaf spot. Later reports by Alexander *et al.* (2), Locke (8), and Andrus and Reynard (3) have shown that resistance to Septoria may be found in certain wild species of *Lycopersicon*. Andrus and Reynard (3) have recently reported on the inheritance of resistance obtained from *L. esculentum* var. Targinnie Red, showing that resistance is inherited as a simple dominant. The actual source of resistance in Targinnie Red is uncertain but Andrus and Reynard indicate that it may have been introduced as a result of natural out-crossing.

METHODS

Heavy sporulation of *Septoria lycopersici* was obtained on potato-dextrose agar. Eight-ounce prescription bottles containing about 25 cc. of agar were inoculated with a concentrated suspension of spores and incubated at room temperature (approximately 22° C.) for 2 to 3 weeks.

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Only inoculum from freshly sporulating cultures gave dependable results. Sporulating cultures were flooded with water and the surface was rubbed with a glass rod. The liquid was withdrawn and agitated in a Waring Blendor for three minutes, strained through cheese cloth, diluted, and applied to the plants by means of a compressed air atomizer similar to that described by Alexander (1). No attempt was made to standardize the dilution. In practice, 12 cultures were usually diluted with 2 qt. of water and used to inoculate 2000 plants. No attempt was made to use a single isolate of the fungus throughout the experiments. Isolations were usually made from field material each summer by means of tissue platings and the resulting cultures used as inoculum for the winter testing program. No study was made to determine possible pathogenic variability of the cultures.

Plants to be tested were transplanted in the cotyledon stage and inoculated when the plants had four true leaves. Immediately after inoculation, the plants were covered with two thicknesses of muslin and incubated in a saturated atmosphere for 40 hr. at about 70° F. Resistant selections have been made only from greenhouse-inoculated plants. The greenhouse method has decided advantages over field testing in that conditions are relatively constant, dependence upon suitable weather is obviated, and the size of field plots can be reduced since only resistant selections need be transplanted.

Crosses were made by pollination of emasculated flowers. Since the greenhouses used were essentially insect-proof, no additional precautions were necessary and it was found by experimentation that natural cross-pollination of flowers emasculated and with corollas removed did not occur in the field.

DISEASE-REACTION TYPES

Andrus and Reynard (3) recognized two types of lesions. The spots produced on susceptible hosts were designated as "Type A" and those on resistant hosts as "Type B." We have encountered similar reaction types and, after early attempts to distinguish intermediates proved unsuccessful, selections were made on the basis of either susceptible or resistant reaction.

On susceptible plants the lesions are first observable as small, circular, grayish, watersoaked spots. These enlarge and develop gray to white centers containing many pycnidia. On the resistant plants the spots are small, reddish brown even when only pinpoint in size, and only occasionally have a small gray center with few or no pycnidia. Both susceptible and resistant reactions appear in about 6 days after inoculation. All records were made 14 days after inoculation. While there was some variation among the lesions in size, color, and number of pycnidia, the two classes, susceptible and resistant, were usually readily distinguishable at all ages. Measurements of the diameters of lesions and counts of the number of pycnidia were made using an F_2 population involving *Lycopersicon hirsutum* as the

source of resistance. The diameters of lesions of the susceptible type varied from 2.5 mm. to 4.5 mm. (average 3.28 mm.) and the number of pycnidia from 11 to 32 per lesion (average 20.66). In resistant plants of the same progeny the diameters varied from 1.5 to 2.5 mm. (average 2.07 mm.) and the number of pycnidia from 3 to 12 (average 6.0). The results are not in complete agreement with those of Andrus and Reynard (3), but their readings were taken seven days after inoculation.

ACCESSIONS TESTED

In seeking sources of resistance to *Septoria lycopersici* all available species of *Lycopersicon*, including many variants of *L. esculentum*, were either observed in the field (2) or tested in the greenhouse. Without exception

TABLE 1.—*Reaction of accessions[a] of four species of Lycopersicon to infection by Septoria lycopersici*

Species	Resistant	Susceptible	Segregating
L. glandulosum	126435, 126438 126439, 126440 126441, 126443 126448	126450, 126949	
L. hirsutum	126445, 126446 126447, 126936 127826	126449, 127827 127828	
L. hirsutum var. *glabratum*		129157, 134417 134418	
L. peruvianum	126431, 128656		126926, 126928 126929, 126930 126944, 126945 126946
L. peruvianum var. *dentatum*	127832	126935, 127830 127831, 128646 128647, 128650 128660, 128661 128663, 129021	128645, 128652 128653, 128655
L. peruvianum var. *humifusum*	127829		
L. pimpinellifolium		118403, 118409 118784, 126433 126924, 126932 126934, 126937 126939, 126941 126948, 126951 126953, 126954 127805, 127806 127807, 127833 129105, 129018	

[a] Accessions designated by P. I. numbers.

L. esculentum L. and *L. pimpinellifolium* (Jusl.) Mill., were susceptible. Certain accessions of *L. glandulosum* C. H. Mull., *L. hirsutum*, and *L. peru vianum* were found to be highly resistant. None of the species, however, was uniformly resistant. *L. cheesmanii* Riley has not been available. The reactions to *Septoria* of accessions of *L. glandulosum*, *L. hirsutum*, *L. peruvianum*, and *L. pimpinellifolium*, listed according to the Plant Introduction numbers, are given in table 1.

INHERITANCE OF RESISTANCE TO SEPTORIA LYCOPERSICI FROM LYCOPERSICON HIRSUTUM CROSSES

Wright and Lincoln (16) reported that several accessions of *L. hirsutum* had proved to be highly resistant to Septoria and that twelve F_1 plants of the cross *L. esculentum* var. Rutgers × *L. hirsutum* P.I. 126445 had been obtained. The material which has been developed as resistant to Septoria at this station is derived from advanced generations of backcrosses to these F_1 plants.

F_1 generation.—Twelve F_1 plants were obtained from the cross of *L. esculentum* var. Rutgers × *L. hirsutum* P.I. 126445. All plants were resistant to Septoria, indicating that resistance is dominant.

TABLE 2.—*Segregation for Septoria resistant and susceptible plants in 15 backcross populations*

Pedigree	Susceptible	Resistant	χ^2 for fit to 1 : 1 ratio
4079–474–1–1–3–2B–B	14	15	0.34
−3B–B	33	24	1.42
−5B–B	21	18	0.23
−6B–B	44	45	0.01
−7B–B	39	47	0.74
−8B–B	19	23	0.38
−9B–B	35	31	0.24
−10B–B	39	61	4.84ᵃ
−4–1B–B	15	13	0.14
−4B–B	21	29	1.28
−5B–B	26	31	0.44
−6B–B	42	42	0.0
−8B–B	21	21	0.0
−9B–B	42	38	0.20
−10B–B	27	32	0.41
Total (df = 15 P = 0.88) (13)	438	470	10.74
Total omitting eighth progeny (df = 14 P = 0.97)	399	409	5.63

ᵃ Significant deviation from expected 1 : 1 ratio at the 5 per cent level.

Backcross generation.—A resistant segregate from the first backcross population was self-pollinated for three generations at which time two selections homozygous for resistance were backcrossed to Rutgers in the greenhouse. F_1 plants of this backcross were again backcrossed to Rutgers. Fifteen backcross progenies were tested for resistance in the greenhouse, and counts on these populations are given in table 2.

Tests in the F_1 plants had shown that resistance was dominant. An inspection of these backcross data shows approximately equal numbers of resistant and susceptible plants, indicating that resistance is controlled by a single factor. Only one of the 15 populations deviated significantly from this hypothesis.

F_2 generation.—In a series of tests involving 976 plants in 58 F_2 populations, each containing about 18 plants, 710 were resistant while 266 were

susceptible. These observations do not deviate significantly ($\chi^2 = 2.64$) from a 3 : 1 ratio which would·be expected for the segregation of a single dominant gene in an F_2 population.

RESISTANCE FROM A CROSS OF RUTGERS WITH LYCOPERSICON PERUVIANUM

In 1943 a cross of *L. esculentum* var. Rutgers by the green-fruited *L. peruvianum* P.I. 126944 was obtained. The resistance of this accession appears to be the greatest of all material tested. A single F_1 plant (Fig. 1)

Fig. 1. Fruit, flowers, and leaf of the F_1 plant of *Lycopersicon esculentum* var. Rutgers × *L. peruvianum* P.I. 126944.

was obtained which proved to be resistant to Septoria. Although its necrotic lesions were larger than lesions on the resistant parent, no pycnidia were observed. F_2 seed was not obtained until 1947, when a large block of F_1 plants, taken to the field as cuttings, produced about 1500 seeds. In an F_2 population of 493 plants tested for resistance to Septoria, 371 plants proved resistant while 122 plants were susceptible. These data indicate that resistance is controlled by a single dominant factor, the χ^2 value of these data fitting a 3 : 1 hypothesis being $\chi^2 = 0.02$.

Despite the high degree of resistance of the *L. peruvianum* parent and the fact that the F_1 plant has been extremely difficult to infect, none of the

F_2 population proved to possess a type of resistance equal to that of the resistant parent. In fact, the lesions (Fig. 2) produced on the F_2 population do not differ significantly from progenies deriving their resistance

FIG. 2. Septoria reaction of resistant F. segregants of the cross, *Lycopersicon esculentum* var. Rutgers × *L. peruvianum* P.I. 126944.

from *L. hirsutum*. Since attempts to cross plants involving the two sources of resistance have been unsuccessful, it has not been possible to determine the identity of the two factors.

IDENTITY OF RESISTANCE FROM LYCOPERSICON HIRSUTUM AND L. ESCULENTUM VAR. TARGINNIE RED

Andrus and Reynard (3) published on the inheritance of Septoria resistance of material derived from the *L. esculentum* var. Targinnie Red and showed that resistance was inherited as a single dominant factor. In an effort to determine whether the factor for resistance obtained from Targinnie Red was the same as the factor from *L. hirsutum*, seed was obtained from Dr. C. F. Andrus of the U. S. Regional Vegetable Breeding Laboratory at Charleston, S. C., and intercrosses were made between homozygous resistant selections from each source. Ten F_1 plants proved to be uniformly resistant and were grown to maturity. The F_2 population of 426 plants was found to be uniformly resistant to Septoria. This indicates that the genes for resistance from the two sources are identical. In fact, the ultimate source of resistance may be the same since Andrus and Reynard (3) state that the Targinnie Red "apparently had become admixed with natural out-crosses so that many segregants were definitely 'non-*esculentum*' in type."

EXPLANATION OF A PRESUMED CROSS BETWEEN BALTIMORE AND LYCOPERSICON
PERUVIANUM VAR. HUMIFUSUM

On the basis of field reaction it was observed that one green-fruited accession, *L. peruvianum* var. *humifusum* P.I. 127829, was extremely resistant to defoliation by Septoria. Crosses of this accession with Indiana Baltimore were attempted in 1939 by Wright (15), and in 1940 Wright and Lincoln (16) reported that two F_1 plants had been obtained that were susceptible to Septoria, indicating that resistance was recessive. Approximately 2000 F_2 plants were grown and field tested in 1941. All plants were susceptible to Septoria and no segregation for any plant character was noted. It seems likely that the presumed F_1 plants resulted from contaminant seed of some *L. esculentum*-like accession that was introduced accidentally into the seed packet or seedling row and certainly was not a hybrid between the parents reported or between any other green-fruited species by a red-fruited species.

MISCELLANEOUS OBSERVATIONS

In 1943 cuttings of the F_1 hybrid between Michigan State Forcing and *Lycopersicon peruvianum* of Smith (12) were provided by J. R. Shay of the University of Arkansas. Seven F_2 plants were obtained, all of which were resistant to Septoria.

In 1947 L. J. Alexander of the Ohio Agricultural Experiment Station sent 60 F_2 plants of the cross Globelle × *L. peruvianum* var. *humifusum* and F_1 seed of 55 different F_2 plants. The F_2 plants and 2450 F_3 plants were tested with Septoria and found to be segregating for resistance. The F_2 plants and 900 resistant F_3 plants were taken to the field for observation. The resistance of this material, as observed in the greenhouse and in the field, did not appear to be greater than that obtained from *L. hirsutum* and present in selections in the advanced breeding program.

STATUS OF THE BREEDING PROGRAM FOR SEPTORIA RESISTANT VARIETIES

A backcross program with either Rutgers or Indiana Baltimore as the recurrent parent has been used to obtain resistant selections of probable varietal value with the resistance being derived from *L. hirsutum* P.I. 126445. Some of these resistant selections have been backcrossed to the commercial type for six backcross generations. Fruit weight of the field selections in 1947 ranged from 115 to 230 gm. The average weight of Rutgers picked at the same time was 200 gm. and of Baltimore, 180 gm. Plant type was indistinguishable from that of the recurrent variety. Greenhouse tests of these 1947 selections showed that most were still segregating for resistance. The work of making varietal selections and testing their yielding ability and plant and fruit characters under a wide range of conditions will still require several years, although it is not expected that further backcrossing to a commercial parent will be necessary in order to select a variety or varieties resistant to Septoria.

No test has been made of the degree of resistance that may be expected in the field once varieties with the present degree of Septoria resistance are introduced. Spore production on resistant plants has been reduced to about 30 per cent of normal, perhaps with a slight delay in the time of sporulation. More lesions are required on a resistant leaf to cause defoliation than on a susceptible leaf. Fruit load and earliness are important factors influencing defoliation, and to date homozygous resistant selections that have the same maturity and yield as commercial varieties have not been available for field tests. It is believed that when resistant varieties are available, the defoliation peak will be delayed by a period of from 2 to 4 weeks, resulting in a reduction of losses.

DISCUSSION

The inheritance of resistance to *Septoria lycopersici* derived from *Lycopersicon hirsutum* and *L. peruvianum* has been shown to be governed by a single dominant gene. This agrees with the published work of Andrus and Reynard (3) who used *L esculentum* var. Targinnie Red as their source of resistance. Although this gene has been called dominant it is to be noted that the size of the lesions and the number of pycnidia present in the F_1 is greater than in the resistant parent. Segregates in advanced generations have not been obtained that equal the resistance of the resistant parent. A possible explanation of this loss of resistance would be loss of modifying factors, although the fact that none of the 493 F_2 plants of the Rutgers by *L. peruvianum* cross possessed resistance equal to that of the resistant parent, detracts from this hypothesis. On the basis of Fisher's (5) theory of dominance, resistance equal to the resistance found in the species material might never be obtained. Varieties that have a fair degree of resistance to *Septoria lycopersici* seem to be assured but may be of temporary value to the tomato industry. The work of Wellhausen (14), Lincoln (7), and Mills (9) on pathogenic changes as influenced by host resistance indicates that, as long as the resistance of the host is not great enough to prevent a successful initial infection, genetic changes affecting pathogenicity may occur in the selective environment of the host and, if more adapted, be preferentially increased. When varieties with the degree of resistance now being developed are introduced, this theory will have an applied test, since such varieties will be subjected each season to primary infection from the pathogen overwintering from the previous year and to a variable number of secondary infection cycles from spores produced on resistant plants.

SUMMARY

Work on developing tomato varieties resistant to *Septoria lycopersici* and the inheritance of resistance is reported.

The reaction to Septoria is reported for 66 Plant Introduction accessions representing four species of the genus *Lycopersicon* other than *L.*

esculentum. Resistance was found in certain collections of the species *L. glandulosum, L. hirsutum,* and *L. peruvianum.*

Resistance for this breeding program was derived from *L. hirsutum* P.I. 126445. It was inherited as a dominant single gene that appears to be identical with the *Se se* factor for Septoria resistance obtained from *L. esculentum* var. Targinnie Red by Andrus and Reynard (3).

The F_2 population derived from the cross *L. esculentum* var. Rutgers × *L. peruvianum* P.I. 126944 also segregates for Septoria resistance on the basis of a monofactorial, dominant gene.

A cross of *L. esculentum* and *L. peruvianum* var. *humifusum* reported earlier from this station as having recessive Septoria resistance is discussed. It is believed likely that the presumed F_1 plants actually were seedlings resulting from contaminant seed.

The breeding program has progressed through six backcross generations to the stage where desirable varietal selections can be made.

Introduction of varieties having the resistance now being worked may be of only temporary value in controlling this disease. It was noted that no selection in the advanced generations of the breeding work has resistance equal to that found in the resistant parent.

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TRENDS IN THE POPULATION OF PATHOGENIC BACTERIA WITHIN LEAF TISSUES OF SUSCEPTIBLE AND IMMUNE PLANT SPECIES[1]

WILLIAM B. ALLINGTON[2] AND D. W. CHAMBERLAIN[3]

The host-parasite interaction in the case of bacterial diseases of plants has been the subject of much interest and speculation. With the exception of certain specific types, *e.g.* the gall producers, most of the information is concerned with the distribution within the tissues or with the effect of enzyme systems and toxins of the parasite upon the host.

When plants are repeatedly inoculated with bacteria and no disease symptoms develop, the host is commonly judged immune or the bacteria nonpathogenic. It is assumed, of course, that the plants are incubated in every case after inoculation under conditions approximating their natural growing conditions. Johnson[4] has shown, for example, the effect of incubation under conditions which maintain watersoaked tissue, a situation rarely observed in nature.

It has been generally accepted that disease symptoms due to bacterial leaf infection are correlated rather closely with bacterial multiplication in the intercellular spaces. A satisfactory quantitative method of measuring this multiplication, however, has never come to the attention of the authors. Preliminary results[5] have indicated the possibilities of a method whereby leaves are watersoaked by forceful spraying and inoculated with standard bacterial suspensions, and the bacterial population within the tissues is determined by the usual plating-out techniques. This article describes the further development of this method and in addition presents data in regard to the dynamics of an immune and susceptible reaction in each of two hosts.

EXPERIMENTAL MATERIALS AND METHODS

Two host plants were selected for this investigation, bean (*Phaseolus vulgaris* L., var. Red Kidney) and soybean (*Glycine max* (L.) Merrill, var. Bansei). The plants were grown in the greenhouse. The bacterial parasites employed were highly virulent isolates of two common leaf spot

[4] Johnson, J. Relation of watersoaked tissues to infection by *Bacterium angulatum* and *Bacterium tabacum* and other organisms. Jour. Agr. Res. [U.S.] 55: 599–618. 1937.

[5] Allington, William B. Wildfire disease of soybeans. Phytopath. 35: 857–869. 1945.

organisms, *Xanthomonas phaseoli* (E. F. Sm.) Dowson, the cause of common bacterial blight on bean, and *Pseudomonas glycinea* (Coerper) Stapp, the cause of bacterial blight on soybean. Care was taken to select two parasites each of which is normally considered to be nonpathogenic on one of the hosts.

Inoculation of bean and soybean with *Pseudomonas glycinea* was accomplished by directing a forceful spray of tap water against the lower surface of attached leaves until the whole leaf area appeared to be water-soaked. The leaves were immediately submerged in a standard suspension of bacteria (8 million bacteria per ml. from a 24-hr. agar culture) for 1 min., exposed to the air for 3 min., and then washed through 5 beakers of sterile water. After this treatment the leaves were untouched except for sampling. Inoculation of bean and soybean with *Xanthomonas phaseoli* was by water-soaking the leaves with the bacterial suspension directly by means of an atomizer. The atomizer used in this work was designed with an electrically driven blower which impels the finely divided liquid with considerable force. The concentration of inoculum, washing procedure, *etc.*, were the same as with *Pseudomonas glycinea*. Repeated tests proved that the leaves were washed adequately to remove bacterial cells adhering to the exterior leaf surfaces. In each test, leaflets of similar position and age (usually in the first trifoliate) were selected on each of 6 plants of a species.

The population of bacteria within the intercellular spaces was determined daily. Disks 4 mm. in diameter were cut from inoculated leaves with a sharp cork borer. Six disks from each species were then thoroughly macerated in 10 ml. of sterile water and allowed to stand for 1 hr. with intermittent shaking. Although the shaking was not standardized, it was as uniform as possible for all the samples from the two host species which were tested simultaneously. Appropriate dilutions were made in sterile water and 1 ml. of the ultimate suspension was added to each of 8 Petri dishes. Wernham's buffered potato-dextrose agar[b] was then added immediately and thoroughly mixed. Colony counts in the culture plates were made in 4 to 5 days in the case of *Pseudomonas glycinea* and 6 to 7 days in the case of *Xanthomonas phaseoli*. The first leaf samples were always taken within 1 hr. after inoculation and subsequent samples were taken at daily intervals. Sampling usually continued for at least 6 days after inoculation. In the case of a congenial host-parasite combination and unusually favorable temperature conditions in the greenhouse, the leaves sometimes withered earlier than 6 days, making further determinations inadvisable.

<div align="center">RESULTS</div>

Pseudomonas glycinea in the leaves of bean and soybean. The results of the daily platings are shown in table 1. Although considerable vari-

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[a] Composed of: potatoes, 200 gm.; dextrose, 20 gm.; agar, 18 gm.; KCl, 0.20 gm.; NaNH$_4$HPO$_4$, 0.50 gm.; NaH$_2$PO$_4$, 0.17 gm.; water, 1000 cc.

TABLE 1.—*Multiplication of Pseudomonas glycinea (Coerper) Stapp in bean and soybean leaves after inoculation by watersoaking*

Time after inocu- lation (days)	Average number of bacteria per 4-mm. leaf disk							
	Test 1		Test 2		Test 3		Test 4	
	Bean	Soybean	Bean	Soybean	Bean	Soybean	Bean	Soybean
0	950	1,783	417	400	63	63	21	21
1	2,500	13,166	3,933	2,700	125	166	55	110
2	6,000	274,833	2,600	7,666	633	3,966	267	833
3	35,000	2,610,000	19,166	181,666	5,166	6,333	550	8,333
4	2,083	881,666	73,333	2,416,666	1,050	4,166	3,333	13,833
5	37,833	13,416,666	24,683	5,850,000	2,283	125,000	1,383	10,000
6			85,833	10,416,666	3,133	750,000	550	8,333
7			84,916	15,666,666	417	20,833	550	2,833
17			155,366					
32			114,266					

ability exists, it is evident that there are definite trends in the bacterial populations depending on the host   In soybean, the susceptible host, multiplication of *Ps. glycinea* was comparatively rapid.   In bean, the non-susceptible host, bacterial multiplication was very definite but at a much slower rate than in soybean.   In tests 1 and 2 these differences were especially striking.   Tests 3 and 4 showed the same general trends but excessively high greenhouse temperatures inhibited the progress of infection. Bacterial blight of soybean is known to be favored by cooler temperatures

The numbers of bacteria entering the leaf tissues at the time of inoculation varied greatly between tests.   No consistent difference in this respect was noted between hosts.

In the case of bean inoculated with *Pseudomonas glycinea* there were no symptoms of the disease in spite of the multiplication and persistence of the organism.   It was notable that in test 2 (Table 1), determinations

TABLE 2.—*Multiplication of Xanthomonas phaseoli (E. F. Sm.) Dowson in bean and soybean leaves after inoculation by watersoaking*

Time after inocu- lation (days)	Average number of bacteria per 4-mm. leaf disk							
	Test 1		Test 2		Test 3		Test 4	
	Bean	Soy- bean	Bean	Soy- bean	Bean	Soy- bean	Bean	Soy- bean
0	246	76	2,026	279	3,041	3,735	1,355	390
1	1,116	296	4,375	1,960	14,700	6,405	73,050	7,061
2	4,033	428	401,866	28,913	242,333	33,366	946,000	49,350
3	188,333	1,850	10,533,333	53,966	25,360,666	166,333	91,633,333	290,166
4	1,646,666	9,666	33,833,333	149,783	49,166,666	10,833	165,333,333	249,166
5	600,000	5,333	13,550,000	602,450	147,000,000	122,833		854,166
6	14,783,333	8,000	164,666,666	278,300				410,333
7	112,833,333	6,166						
14		3,183						
16		3,250						
28		955						

made 32 days after inoculation revealed substantial numbers of bacteria in the leaf tissues.

Xanthomonas phaseoli in leaves of bean and soybean. The results with *X. phaseoli* (Table 2) show much less variability than those with *Ps. glycinea* (Table 1). A reciprocal condition exists, however, with the two hosts. In the case of soybean, multiplication occurred but proceeded at a slower rate. No symptoms were produced and the organism persisted in substantial numbers in the inoculated leaves in one of the tests for at least 28 days.

Multiplication of this organism in the leaf of bean was very rapid, as expected. Each test was discontinued the day the leaves withered from the effects of the infection. Increasing greenhouse temperatures late in the spring accounted for the acceleration of bacterial multiplication and leaf tissue collapse shown progressively in tests 1–4 (Table 2).

DISCUSSION

Various investigators have observed the persistence of nonpathogenic bacteria within host tissues. The study of the behavior of pathogenic forms in immune species or resistant varieties by the method described in this article stimulates new lines of thought bearing on the dynamics of disease resistance. It has been shown in this study, for example, that *Pseudomonas glycinea* and *Xanthomonas phaseoli* multiply when introduced into the leaf tissues of hosts normally considered to be immune. The rates of multiplication in the susceptible and immune species do not differ greatly until the second or third day after inoculation. At this time large populations of bacteria are present in the tissues. This would indicate that the immune reaction is inoperative until the bacteria in the intercellular spaces have an effect upon the adjoining living cells. Further investigations will be necessary to conclusively prove this hypothesis.

The knowledge that at least some pathogens can live and multiply in immune hosts, under natural conditions, has an influence upon the theories of epidemiology, the dynamics of disease resistance, and the adaptation of pathogens to new hosts.

SUMMARY

A method of studying the multiplication of bacterial pathogens within plant leaves is described. By the use of this method two pathogens were studied in immune and susceptible hosts. It was found that multiplication, presumably in the intercellular spaces, was initiated about equally in both the immune and susceptible host. After a time the inhibitory effect of the noncongenial host became apparent when the bacterial population was found to increase less rapidly or to decrease markedly. The popula-

tion within the congenial host continued to increase until destruction of the tissues occurred.

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NATURAL SPREAD OF PHONY DISEASE TO APRICOT AND PLUM[1]

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Artificial transmission of phony disease of peach to other *Prunus* species (almond, common apricot, Japanese apricot, and plum) by means of root-to-root graft inoculations was reported in 1933.[4] The present paper describes observations and experiments establishing the fact of natural spread of the disease to two species of apricot and three species of plum. The work was performed at the U. S. Horticultural Field Laboratory, Fort Valley, Georgia, where phony-affected peach trees were growing and natural spread of the disease in peach was known to be taking place.

After long exposure to natural infection, which is described for each species, a representative number of trees was tested for presence of phony disease virus in the roots by indexing to peach. For this purpose, normal peach nursery trees of a commercial variety were obtained in a territory free of phony disease. On the tap root of each index tree was grafted a root piece 5 or 6 in. long, from one of the trees to be tested. The grafting was done in winter, and the index trees were planted 3 ft. apart in nursery rows. After an incubation period of 18 months or more following graft inoculation, the peach index trees were examined to determine whether the disease had been transmitted. Uninoculated peach nursery trees, interspersed among the index trees in the plantings, served as checks to determine the hazard of natural spread of phony disease in the index test plots. Tests demonstrating that 5 *Prunus* species had contracted phony disease through natural spread are summarized in table 1.

COMMON APRICOT

In March 1932, the Division of Plant Exploration and Introduction supplied small seedlings of common apricot, *Prunus armeniaca* L., under 13 Plant Introduction numbers. The seeds for the seedlings were obtained at several locations in southern Europe and northern Africa. These seedlings were planted in the experimental nurseries at Fort Valley, Georgia. For each P. I. number, 10 trees received root grafts from phony-diseased peach trees, and 10 trees were used for checks and for later observation on natural spread of the disease to apricot.

TABLE 1.—*Data showing natural spread of peach phony disease to other Prunus species, Fort Valley, Georgia*

Prunus species	Trees indexed to determine natural infection	Root pieces indexed per tree	Peach index trees[a]		Natural spread to peach check trees in the index-tree test plot			
			Trees on which root graft survived	Developed phony	Check trees	Developed phony	Remained normal	Natural spread
	Number	Number	Number	Percent	Number	Number	Number	Percent
P. armeniaca ...	6	5	24	100				
P. mume	6	3	18	100	59	10	49	16.9
P. hortulana	12	5	60	100				
P. mexicana	6	5	30	100	50	0	50	0.0
P. angustifolia	5	3	15	100	128	0	128	0.0

[a] As here used, a peach index tree is a nursery peach tree on the tap root of which is grafted a root piece from another tree. If phony disease virus is present in the root piece, the index tree will develop the disease after an incubation period of about 18 months.

After an incubation period of 18 months, many of the artificially inoculated trees, representing all of the P. I. numbers, developed dwarfed growth and profuse lateral branching typical of phony disease (Fig. 1, A and B), whereas the check apricot trees were normal in appearance (Fig. 1, C and E). However, as the years passed it was noted that an increasing number of the check trees also developed symptoms of the disease (Fig. 1, D).

On February 3, 1938, root-graft inoculations to peach index trees were made to determine whether the dwarfed condition in the apricot was due to the presence of phony disease virus.

In the case of the artificially inoculated apricot trees, subinoculations to peach were made from 6 trees, all of which for several years had shown

FIG. 1.   Common apricot trees of same age: A and B, phony-affected as a result of root-graft inoculation from diseased peach; C and E, normal, bearing no root graft; D, bearing no root graft, but phony-affected as a result of natural infection.

symptoms resembling those of phony disease. Thirty peach index trees, in lots of 5 for each apricot tree, received graft inoculations with apricot roots. By October 2, 1939, after an incubation period of 20 months, each of 21 of the peach index trees bore a living apricot root graft, and of these, 18 trees, or 85.7 percent, had developed positive symptoms of phony disease (Fig. 2, A).

For confirmation of the suspected natural transmission of phony disease to apricot, 6 apricot trees showing symptoms of the disease without artificial inoculation (Fig. 1, D) were indexed by root grafts to peach. Five peach index trees were used for each apricot tree. When the test was terminated on October 2, 1939, after an incubation period of 20 months, all of the 24 index trees on the tap root of which the apricot root

graft survived, showed typical symptoms of phony disease (Fig. 2, A, and Table 1).

Root-graft inoculations to peach index trees showed the virus to be present in 2 or 3 of the 5 apricot root pieces tested from each of 5 normal-appearing apricot trees   Based on experience with peach, this indicated that these apricot trees were in the incubation stage of phony disease.
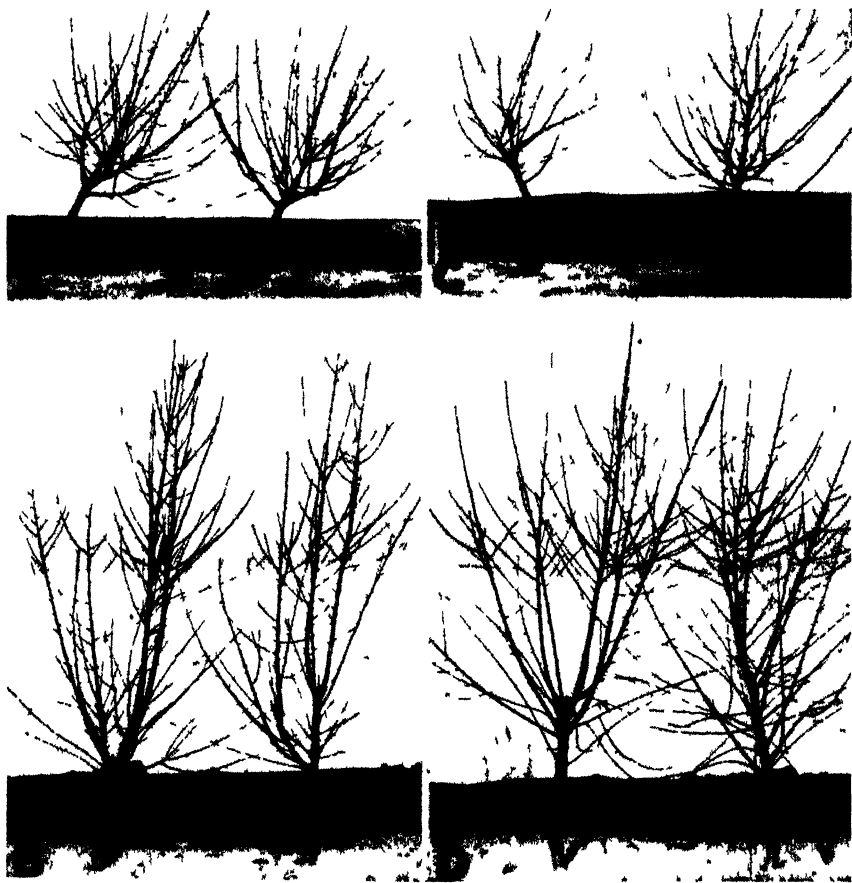
FIG. 2   Elberta peach trees of same age: A, index trees, phony affected as a result of root graft inoculation from diseased common apricot; B, normal noninoculated check trees; C, index trees, phony affected as a result of root graft inoculation from diseased Japanese apricot; D, normal index trees, bearing root graft from normal Japanese apricot tree

For each of 2 other normal-appearing apricot trees similarly tested by grafting on the roots of peach index trees, 4 out of 5 apricot roots survived and all of the index trees remained normal.

Natural spread of phony disease to common apricot was evident from these tests, and symptoms in the naturally infected apricot trees were identical with symptoms in the apricot trees that were inoculated by root grafts from phony-diseased peach trees.

JAPANESE APRICOT

In March 1929, 54 seedling nursery trees of Japanese apricot, *Prunus mume* Sieb. & Zucc., P. I. No. 26886, were planted 3 feet apart in a nursery row at Fort Valley, Georgia, where they were grown for 9 years in the vicinity of a high incidence of phony-diseased peach trees and were frequently observed for symptom manifestation of the disease.

In 1934, 5 years after the trees were planted, they had attained a height of 9 to 10 ft. with profuse interlaced lateral branches, typical of the species. As would be expected in such a group of seedlings, variations in vigor, leaf size, and seasonal response among individuals were noted, but none of the trees had the excessive dwarfing associated with phony disease in peach and common apricot. At this time (1934) the entire group of trees was pruned to an even height of about 5 ft., so that the row presented the appearance of a clipped hedge. This type of pruning, when applied to peach or to common apricot grown under similar conditions, is an effective means of forcing symptoms of phony disease in trees bearing the causative virus. Following this severe pruning, the *mume* trees grew to a height of 10 to 12 ft. and did not manifest identifiable symptoms of the disease. To determine whether some of the trees were symptomless carriers of the virus, root-graft inoculations were made to peach. Three peach index trees were used for each of the 54 *mume* trees, thus making a total of 162 index trees.

The grafting was performed in February 1938. In August 1939, after an incubation period of 19 months, final data were taken on the condition of the grafts and the behavior of the index trees. For 6 *mume* trees, each peach index tree, 18 in all, bore a living *mume* root graft and showed phony disease symptoms (Fig. 2, C). For 20 other *mume* trees, each peach index tree, 60 in all, bore a living *mume* root and showed no symptoms of phony disease (Fig. 2, D). These results established the fact that Japanese apricot is susceptible to natural infection by phony-disease virus.

The results of the index test on the remaining 28 *mume* trees are not discussed here because of variability among the index trees due to failure of one or more of the *mume* root grafts, or to differences in incubation stages, in which some *mume* roots transmitted the disease to peach and some did not.

PRUNUS HORTULANA AND PRUNUS MEXICANA

Seeds of the Hortulan plum, *P. hortulana* Bailey, and of the big-tree plum, *P. mexicana* S. Wats., were obtained from sources outside the phony-disease area, and were planted at Fort Valley, Georgia, in January 1930.

In the resultant blocks of several hundred seedlings of each species, no mechanical inoculations were made, but 8 years later (July 1938) some of the seedlings displayed growth and leaf symptoms suggestive of phony

disease. At that time, 17 such trees of *P. hortulana* and 15 of *P. mexicana* were selected for indexing to peach in order to determine whether they had contracted phony disease from natural inoculations.

Five index trees were employed for each plum tree, which made a total of 85 index trees for *P. hortulana* and 75 for *P. mexicana*. The grafting was performed on January 28, 1939, and the trees were planted in the experimental nursery the following day. Interspersed among the 160 index trees in the planting were 50 noninoculated peach check trees.

In late August 1940, after an incubation period of 19 months, the peach index trees were examined for symptoms of phony disease. For 12 of the 17 *P. hortulana* trees, each of 60 peach index trees bore a living plum root and showed positive symptoms of phony disease. For 6 of the 15 *P. mexicana* trees, each of 30 peach index trees bore a living plum root and showed positive symptoms of phony disease. The results of the index test on the remaining trees of these two plum species are not discussed here because of variability in the index tree lots due to failure of one or more of the plum root grafts, or to differences in incubation stages,in the plum, so that some of the roots transmitted the disease to peach and some did not. All of the noninoculated peach check trees were normal. It was proved by these tests that *P. hortulana* and *P. mexicana* are subject to natural infection by phony disease virus.

PRUNUS ANGUSTIFOLIA

Natural spread of phony disease to two apricot species and to two plum species having been established, the following experiment was performed to determine whether wild plums in the vicinity of heavily diseased peach orchards also might harbor the causative virus. A thicket of Chickasaw plum, *P. angustifolia* Marsh, growing at the edge of a field near Oglethorpe, Georgia, from which a commercial peach orchard showing very high incidence of phony disease recently had been removed, was chosen as a source of representative material for the test.[5]

In January 1941, 30 plum seedlings from this thicket were transplanted to the experimental nursery at Fort Valley, Georgia, for further observation and for indexing to peach. Three index trees were employed for each of 15 plum trees. Grafting and planting were done on February 13, 1941. The test block consisted of the 45 inoculated peach index trees and 128 noninoculated peach check trees.

The results of the experiment were observed in the summer of 1942. For 5 plum trees, each of 15 peach index trees bore a living plum root and showed positive phony disease symptoms. For 5 other plum trees, each of 15 peach index trees bore a living plum root and showed no symp-

toms of phony disease. The results of the index test on the remaining 5 plum trees are not discussed here because of variability among the index trees due to failure of one or more of the plum root grafts, or to differences in incubation stages in the plum, so that some roots transmitted the disease to peach and some did not. All of the 128 peach check trees were normal at the termination of the experiment. The experiment demonstrated conclusively that Chickasaw plum trees are subject to natural infection with phony disease virus.

SUMMARY

Natural spread of phony disease virus to seedling trees of 2 species of apricot and 3 species of plum, growing near phony-diseased peach trees, was demonstrated in the tests here reported. Symptom manifestation in the infected trees tested was strong in *Prunus armeniaca*, less marked in *P. hortulana* and *P. mexicana*, and highly indefinite in *P. mume* and *P. angustifolia*. Infected trees of the latter two species were virtually symptomless carriers of the virus.

PLANT INDUSTRY STATION
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PHYTOPATHOLOGICAL NOTES

Comparison of Methods for Artificially Inoculating Sugar-Cane Seedlings with the Mosaic Virus.—Bain[1] reported transmission of sugar-cane mosaic by an abrasion technique, using either sand or carborundum as the abrasive. He worked with plants of seedling progenies that differed genetically in their susceptibility to mosaic and thus did not permit accurate comparison of the abrasion with the needle-prick method,[2] which is ordinarily used for artificial transmission of this disease. Since the abrasion technique is more rapid and economical and permits inoculation of younger plants in testing the several thousand seedlings produced each year in the sugar-cane breeding program, it was important to determine whether it is as effective as the needle-prick method in obtaining infection. The two methods were compared in several experiments, using three sugar-cane clones: Louisiana Purple, very susceptible, and Co. 281 and Co. 290, moderately susceptible to mosaic.

TABLE 1.—*The mosaic infection in sugar-cane clones inoculated by abrasion or by needle prick.*

Clone	Plants infected[a]	
	Abrasion method	Needle-prick method
	Per cent	*Per cent*
Louisiana Purple	100	89
Co. 281	89	60
Co. 290	86	44

[a] Differences between methods for each variety significant at the 5 per cent level.

Single-bud cuttings of each were germinated in 4-in. pots of steamed soil in the greenhouse, and when the plants were 3 to 4 weeks old and 4 to 6 in. tall they were arranged in randomized blocks on the benches, with 10 plants in each of the 10 replications of each treatment. Half of the plants were inoculated by needle prick and half by abrasion, using washed, 80-mesh, sea island sand (which other experiments had shown to be more effective than 100-mesh sand, or 80-mesh and 100-mesh carborundum). The results of one experiment are typical of several that were performed (Table 1).

Because of the several advantages of the abrasion method, and the fact that these results showed it to be as effective as the needle-prick technique, it has been adopted for the routine inoculation of sugar-cane seedlings in testing for mosaic resistance. Admittedly, inoculation of very young plants

[1] Bain, Douglas C. The use of abrasives for inoculating sugar-cane seedlings with the mosaic virus. Phytopath. **34**: 844–845. 1944.

[2] Matz, Julius. Artificial transmission of sugar-cane mosaic. Jour. Agr. Res. [U. S.] **46**: 821–839. 1933.

with abrasives may result in infection (and consequent elimination from further consideration) of some clones that would not become infected if inoculated by the needle-prick method, or when exposed to natural infection in the field. This possibility was investigated by inoculating by both techniques 10 young plants, grown from cuttings of each of the following sugarcane varieties that have shown only rare or no cases of mosaic in commercial culture or in extensive experimental field plantings, and which are difficult if not impossible to infect artificially by the needle-prick technique: C. P. 28/19, 29/320, 34/92, 34/120, 36/13, 36/19, 36/105, 36/183, and 43/47. Three plants of C. P. 43/47, two of C. P. 36/183, and one of C. P. 36/13 were infected by the abrasion technique. Two plants of C. P. 36/19 were infected by the needle-prick method. Thus, infection of some resistant clones may result from the artificial inoculation techniques used. However, since more seedlings are usually produced each year in the breeding program than can be handled in the field and some must be discarded anyway, it is believed that elimination of the several thousand that can be infected with mosaic at this early stage is justified.

While the abrasion technique gives better virus transmission than the needle-prick in the inoculation of young plants, in other experiments it was found, as mentioned by Bain, that as plants increased in age and size before inoculation the method became less effective; and that with plants several months old, higher infection was obtained with the needle-prick technique. Inoculating the whorl of unfolding leaflets by the abrasion method gave significantly higher infection than inoculating the completely unfolded leaves.

Experiments were also performed to determine whether it was feasible to inoculate seedlings with mosaic in the germinating flats prior to potting, thereby saving the labor and greenhouse space required for potting the several thousand that are eventually eliminated because of mosaic susceptibility. The unfolding leaflets of the plants in the flats were crushed between small, flat pieces of wood covered with fine sandpaper (which was more rapid than rubbing with the fingers) and then atomized with extracted juice of mosaic-infected plants. Infection comparable with that obtained by other methods was obtained. However, this technique has not been adopted as a routine procedure because it is difficult to avoid uprooting some seedlings, and practically impossible to give uniform treatment to all plants. In some tests a high percentage of infection was obtained when the leaves were clipped back to slightly above the growing point and the plants immediately atomized with infective juice, but at other times no infection resulted from this treatment.—E. V. ABBOTT, United States Sugar Plant Field Station, Houma, Louisiana.

Some Unreported Host Plants of Sugar Beet Mosaic Virus.—In fields of sugar beets in southern California three species of plants have been found naturally infected with sugar beet mosaic that appear not to have

been reported previously as susceptible to this disease. These are *Amaranthus retroflexus* L. (pigweed), *Melilotus indica* (L.) All. (yellow sweet clover), and *Trifolium incarnatum* L. (crimson clover). The disease was transmitted experimentally from sugar beet to these species and to the following seven additional species that appear also to be unrecorded hosts, by means of the aphid, *Myzus persicae* Sulz.: *Beta patellaris* Moq., *Browallia speciosa* Hook., *Nicotiana quadrivalvis* Pursh and var. *multivalvis* (*Lindl.*) Gray, *N. clevelandi* Gray, *Phacelia campanularia* Gray, *Pisum sativum* L. (garden pea, var. Little Gem), and *Samolus parviflorus* Raf. (water pimpernel). The disease was transmitted to sugar beet from plants of each of these species by juice inoculation.

Grafts were made from diseased plants of *Nicotiana clevelandi* and *N. quadrivalvis* to healthy plants of *Datura meteloides* DC. (tolguacha), *D. stramonium* L. (jimsonweed), *Lycopersicon esculentum* Mill. (tomato), *Nicotiana acuminata* (Grah.) Hook., *N. glauca* Grah. (tree tobacco), *N. glutinosa* L., *N. langsdorffii* Weinm., *N. longiflora* Cav., *N. palmeri* Gray, *N. paniculata* L., *N. rustica* L., *N. sylvestris* Speg. and Comes, and *N. tabacum* L. (tobacco, var. Turkish). No evidence of systemic infection was obtained on any of these species and juice from the stock variety failed in all cases to produce infection on sugar beet, whereas inoculation with juice from the scion variety resulted in high percentages of infection in all tests. Therefore, it appears that all of these species are immune from systemic infection by sugar beet mosaic virus.

Other plants were heavily infested repeatedly with *Myzus persicae* from mosaic sugar beet but showed no evidence of infection. These include *Brassica nigra* (L.) Koch (black mustard), *Cucumis sativa* L. (cucumber, var. Long Green), *Lactuca sativa* L. (lettuce), *Medicago sativa* L. (alfalfa), *Melilotus alba* Desr. (white sweet clover), X *Petunia hybrida* Vilm. (petunia), *Phaseolus vulgaris* L. (bean, vars. Kentucky Wonder, Stringless Greenpod, Great Northern, and Red Kidney), *Phytolacca americana* L. (pokeweed), *Plantago lanceolata* L., *Rumex acetosella* L., *Sisymbrium irio* L., *Sonchus asper* L., *Trifolium pratense* L. (red clover), *T. repens* L. (white clover), and *Zinnia elegans* Jacq.

Symptoms of the disease on plants shown to be susceptible consisted of various types of mottling more or less covering the range of mottling found on sugar beet. On *Melilotus indica* chlorosis was limited to the veins and immediately adjacent tissue in some leaves but appeared as various types of mottling in others as shown in figure 1. Plants of *Trifolium incarnatum* were dwarfed and the leaves were deformed and showed some necrosis in addition to mottling and vein chlorosis. A common pattern of mottling with little or no leaf dwarfing and no deformity was produced on *Nicotiana clevelandi* and *N. quadrivalvis*. On *Samolus parviflorus* necrosis was produced first, followed by a certain amount of recovery and mottling. Plants of *Beta patellaris* had dwarfed, mottled, and deformed leaves, but in many instances the disease was restricted to one or a few shoots, other

shoots on the same plant remaining for long periods free of symptoms and of virus. Local necrotic lesions 2 to 4 mm. in diameter were produced on leaves rubbed with juice from diseased plants. These lesions were definite and numerous enough to be useful in studies of properties of the virus. Similar lesions were produced on inoculated leaves of the Detroit Dark Red variety of garden beet.

FIG. 1. Leaves of *Melilotus indica* (yellow sweet clover), showing range of mottling observed on plants attacked by sugar beet mosaic.

Melilotus indica has been found with an appreciable percentage of infection in most locations where it was growing in close association with diseased sugar beets, including plantings near Tucumán and Conesa in Argentina, observed in 1941. *Trifolium incarnatum* used as a cover crop in plots near diseased sugar beets at Hemet, California, was rather heavily infected in 1943. However, it is not believed that these plants or any of the susceptible weed hosts serve as important sources of virus for sugar

beet or garden beet. Evidence indicates strongly that virus is carried into new fields mainly from older diseased beet plants that have been left or planted near new plantings.—C. W. BENNETT, U. S. Sugar Plant Field Laboratory, Riverside, California.

The Length of Tobacco Mosaic Virus Particles from Juice and from Leaf Residue.—Earlier workers have disagreed regarding the relative length of tobacco mosaic virus particles obtained from juice and from the residue after the juice has been extracted. Some evidence has been presented indicating that the particles obtained from the residue are shorter than those from juice.[1,2] Gaw,[3] on the contrary, maintained that virus particles from juice have essentially the same length as those from residue. This question is important in that it may provide significant evidence regarding the mode and site of virus multiplication. We have accordingly attempted to obtain conclusive information regarding the relative length of virus particles from juice and from residue.

The juice virus was obtained by freezing the leaves, adding 3 per cent of the fresh weight of powdered K_2HPO_4 to the frozen leaves, grinding in a meat chopper, and pressing out the juice. The juice was then subjected to alternate low and high speed centrifugation until pure enough for electron microscopy. The residue virus was prepared essentially by the method of Bawden and Pirie.[4] The residue left after the juice was pressed out was washed with four changes of distilled water, and a small portion of the residue was ground in a hand-operated, ground-glass homogenizer. Phosphate-HCl buffer[5] was added during the grinding to produce the best liquid content for grinding and to hold the pH around 7. The preparation was then subjected to alternate low and high speed centrifugation, and the resulting virus was subjected to electron microscopy. Both juice and residue viruses were kept in phosphate-HCl buffer throughout the various centrifugations to prevent aggregation of virus particles. The length of the virus particles was measured on enlarged prints.

Typical results are shown as length distribution curves in figure 1. The virus particles from juice or residue are in two distinct length groups that we have previously called "short group" and "middle group." The "long group" particles that result from end-to-end aggregation of shorter particles were essentially absent in our preparations.

[1] Bawden, F. C., and N. W. Pirie. The separation and properties of tobacco mosaic virus in different states of aggregation. Brit. Jour. Exper. Path. 26: 294–312. 1945.

[2] Crook, E. M., and F. M. L. Sheffield. Electron-microscopy of viruses: I. State of aggregation of tobacco mosaic virus. Brit. Jour. Exper. Path. 27: 328–338. 1946.

[3] Gaw, H. Zanyin. A comparative study of the properties of two strains of tobacco mosaic virus prepared from the sap and from the leaf residues of Turkish tobacco plants. Archiv. f. Gesam. Virusforsch. 3: 347–355. 1947.

[4] Bawden, F. C., and N. W. Pirie. The liberation of virus, together with materials that inhibit its precipitation with antiserum, from the solid leaf residues of tomato plants suffering from bushy stunt. Brit. Jour. Exper. Path. 25: 68–80. 1944.

[5] Rawlins, T. E., Catherine Roberts, and Nedra M. Utech. An electron microscope study of tobacco mosaic virus at different stages of infection. Amer. Jour. Bot. 33: 356–363. 1946.

Each experiment was repeated at least three times, and a relatively large number of particles was measured in each experiment. The results of these experiments are shown in table 1. The results in figure 1 and table 1 show that about 70 per cent of the particles of juice virus are in the middle
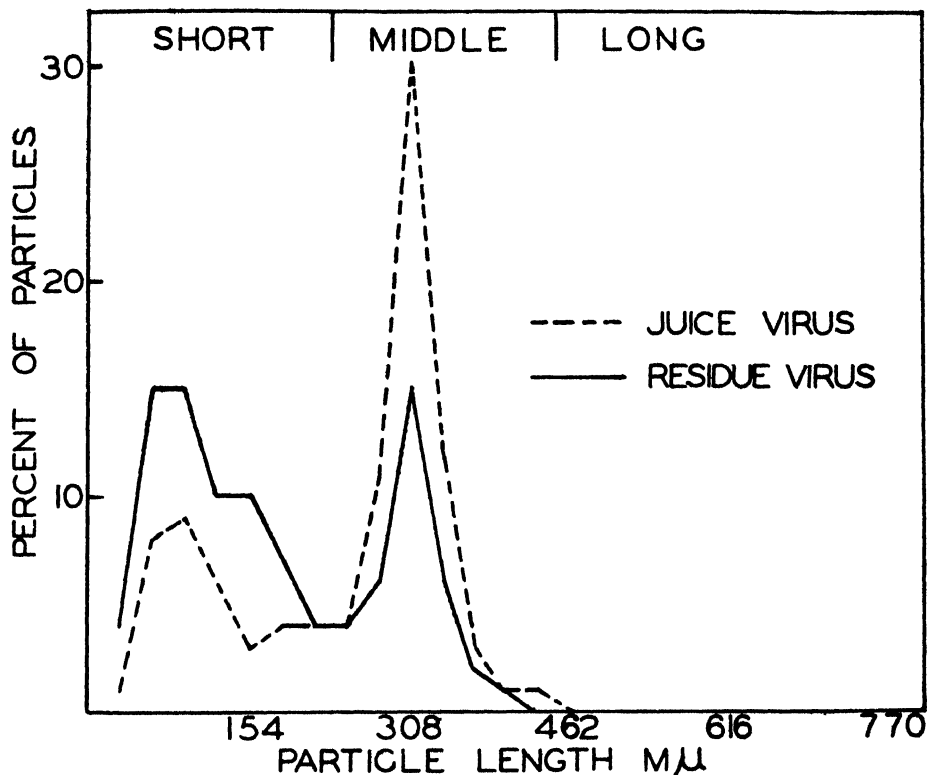
FIG. 1.   Typical length distribution curves of juice and residue viruses.

group having a mode around 300 mμ; about 30 per cent of the juice virus particles fall in the short group. Residue virus, on the contrary, contains about 70 per cent short group particles and 30 per cent middle group particles.

Experiments were conducted to determine whether the high proportion of short group particles in the residue might be caused by the homogenization. Juice virus was homogenized, and healthy residue to which purified juice virus had been added was likewise homogenized. In each case electron micrographs showed that the homogenization did not produce a detectable change in the length of the juice virus particles. These results apparently prove that the high proportion of short group particles in the residue virus is not due to breaking up of virus particles during homogenization.

The results apparently provide conclusive evidence that virus from residue contains a much higher proportion of short group particles than does virus from juice.

Because Gaw[3] concluded that juice virus and residue virus have essentially the same size, we have made careful measurements of the virus particles in his micrographs. We find that 15 per cent to 60 per cent of the particles in his four preparations are longer than 446 mμ. Comparing these results with our table 1, it appears that his preparations were aggregated. This marked aggregation resulting from his methods of preparation apparently accounted for his failure to detect a difference in the length of virus particles in juice and in residue.

The fact that Oster and Stanley[6] obtained a typical juice virus length distribution curve by breaking infected hair cells in distilled water may be due to the short residue virus particles remaining occluded in protoplasm and therefore not being shown in the electron micrographs. The middle group particles, which they observed in abundance, apparently are relatively free in the cell, may escape into the water, and may be readily detected by means of the electron microscope.

TABLE 1.—*Length of tobacco mosaic virus particles obtained from residue and from juice*

Experiment No.	Source of virus	Number of ultracentrifugations	Number of particles measured	Percentage shorter than 231 mμ	Percentage between 231 and 446 mμ long	Percentage longer than 446 mμ
23D	Residue	2	1049	65	35	0
17	Residue	1	128	84	16	0
20D	Residue	3	234	59	36	5
23A	Juice	2	267	35	63	2
43	Juice	1	238	27	60	13
10	Juice	2	828	26	68	6
1	Juice	3	599	27	71	2

Another hypothesis that is in accordance with all of the observed results is that most of the residue virus results from the rupture of chloroplasts during homogenization. The small proportion of short particles obtained by Oster and Stanley from hair cells might be due to the absence of chloroplasts in these cells.

We have studied the infectivity of juice and residue virus and find, as have earlier workers [1,7,8,9] that most or all of the infectivity is in the middle group particles; short group particles show little, if any, infectivity.

Since healthy tissues do not yield any elongated particles detectable by the electron microscope, the available evidence indicates that the short group particles represent a stage of the virus that is noninfective when

[6] Oster, G., and W. M. Stanley. An electron microscope study of the contents of hair cells from leaves diseased with tobacco mosaic virus. Brit. Jour. Exper. Path. 27: 261–265. 1946.

[7] Lauffer, Max A. The sedimentation rate of the infectious principle of tobacco mosaic virus. Jour. Biol. Chem. 151: 627–634. 1943.

[8] Sigurgeirsson, Thorbjorn, and W. M. Stanley. Electron microscope studies on tobacco-mosaic virus. Phytopath. 37: 26–38. 1946.

[9] Oster, Gerald. Studies on the sonic treatment of tobacco mosaic virus. Jour. Gen. Physiol. 31: 89–102. 1947.

removed from the host.—WILLIAM N. TAKAHASHI and T. E. RAWLINS, Division of Plant Pathology, University of California, Berkeley, California.

Peronospora tabacina Adam in Washington State.[1]—In 1891 Spegazzini described *Peronospora Nicotianae* occurring on *Nicotiana longiflora* in Argentina. Later he recorded the occurrence of this fungus on other species of *Nicotiana* native to South America.

In the past 25 to 30 years the blue mold of tobacco has caused severe losses in seed beds in the tobacco-growing regions of the United States and Australia. Clayton and Stevenson[2,3], after considerable study, have concluded that the causal organism of blue mold of tobacco is distinct from *P. Nicotianae* Speg. and should be known as *P. tabacina* Adam. Indeed, they concluded that it is improbable that the "oospores" found in Spegazzini's material are those of a *Peronospora,* since the measurements (76–80 μ) are greater than those recorded for the oospores of any other *Peronospora* species[3]. It is interesting to note that none of the four Spegazzinian specimens studied by Clayton and Stevenson[2] possesses both "oospores" and conidia. Three of the four specimens contained only "oospores," while the fourth bore only conidiophores and conidia. I suggest that *P. Nicotianae* Speg. is a *Nomen confusum,* being based on the conidiophores and conidia of the fungus now generally called *P. tabacina* Adam and on the oosporelike bodies of some other fungus.

Prior even to Spegazzini's first collection (1888), Farlow[4] in 1885 collected a *Peronospora* in California on *N. glauca,* this host being a species which had been introduced from Argentina[4]. However, this is not one of the hosts upon which Spegazzini recorded the presence of his *P. Nicotianae.* At the time it was collected, the California fungus was referred to *P. Hyoscyami* de Bary, a European species which at present is considered to be restricted to *Hyoscyamus niger.* Harkness in 1886 recorded the occurrence of a *Peronospora* on *N. Bigelovii* in Nevada, and recently Stevenson and Archer[5] have reported *P. tabacina* on *N. attenuata* from the same State. However, the identity of all previous collections from western United States has been subject to doubt because they lacked oospores.

A downy mildew recently has been collected on *N. attenuata* in Washington State. The collection was made by J. D. Menzies at Prosser, Benton County, Washington, during the summer of 1946 (WSC-PP No. 15947; portion also deposited in the herbarium at the Bureau of Plant Industry,

[1] Published as Scientific Paper No. 821, College of Agriculture and Agricultural Experiment Stations, Institute of Agricultural Sciences, State College of Washington, Pullman, Washington.

[2] Clayton, E. E., and J. A. Stevenson. Nomenclature of the tobacco downy mildew fungus. Phytopath. 25: 516–521. 1935.

[3] ——————, and ——————. *Peronospora tabacina* Adam, the organism causing blue mold (downy mildew) disease of tobacco. Phytopath. 33: 101–113. 1943. (Other relevant earlier papers are cited therein.)

[4] Farlow, W. G. Notes on some injurious fungi of California. Bot. Gaz. 10: 346–348. 1885.

[5] Stevenson, J. A., and W. A. Archer. A contribution to the fungus flora of Nevada. U. S. Dept. Agr., Pl. Dis. Reptr. 24: 93–103. 1940.

U. S. Department of Agriculture, Beltsville, Maryland). The specimen is of considerable interest since it bears conidiophores and conidia and also contains oospores.

The oospores measure 40–57 μ in diameter, with a mean of 47.2 μ. These oospore measurements fall within the range of those reported by other workers for the oospores of *P. tabacina* Adam, and are very close to those originally given by Adam (35–60 μ, mean 46 μ) in his description of material on *N. Tabacum* from Australia. Of even greater importance is the fact that the exospore definitely places this Washington State collection in the section Effusae of the genus *Peronospora*, while the "oospores" present in Spegazzini's material must, on the basis of the exospore structure, be referred to a species of the section Calothecae, if these structures are considered those of a *Peronospora*. The Washington State collection is therefore identified as *P. tabacina* Adam. Furthermore, this specimen containing oospores supports the opinion that the earlier western collections are also *P. tabacina*.

The Washington State specimen, collected on a rather rare native plant in a non-tobacco-growing region would seem to support the theory that the downy mildew is endemic on the Pacific Coast, were it not for the fact that at least one host susceptible to *P. tabacina,* namely *N. glauca,* had been introduced on the Pacific Coast from South America prior to the first collection of the downy mildew[4]. If this known host introduction is considered, there is still doubt as to whether *P. tabacina* was originally endemic to all three areas (North America, South America, and Australia) where species of *Nicotiana* are native, or whether it has spread from one of these areas to the others.—CHARLES GARDNER SHAW, Department of Plant Pathology, State College of Washington, Pullman, Washington.

VERTICILLIUM WILT OF AVOCADO[1]

GEORGE A. ZENTMYER[2]

In the past 15 to 20 years there have been occasional reports of sudden wilting and collapse of isolated avocado trees in otherwise healthy groves in California. This trouble, which was thought to be connected with a lack of oxygen in the soil, brought about by a sudden saturation of the soil with

FIG. 1. Fuerte avocado tree affected with Verticillium wilt. The entire top wilted suddenly, and the leaves died and turned brown. A few new shoots are developing from the trunk.

[2] Associate Plant Pathologist.

677

water, has been variously termed collapse, asphyxiation, and apoplexy (1, 3). Investigations during the winter of 1947–1948, reported in the present paper, showed that this disease is caused by the fungus *Verticillium albo-atrum* Reink. and Berth.

SYMPTOMS

The symptoms of Verticillium wilt on avocado are similar to those on other woody hosts including maple, elm, apricot, and peach. There is usually a sudden wilting of the leaves on one branch, on one side of a tree, or on an entire tree, followed quickly by death of the leaves (Fig. 1). The leaves turn brown and remain on the tree for several months.

At the time of the initial wilting and "collapse," dark-brown streaks are readily seen in the most recently formed xylem tissue when the bark is peeled from affected branches or from the trunk. Streaks may be abundant, covering most of the circumference of the branch (Fig. 2), or may be few in number.

FIG. 2. Healthy avocado branch (left) and three diseased branches (right), from which bark has been peeled, showing vascular discoloration (brown) caused by *Verticillium albo-atrum*.

Dieback occurs in varying degrees, and occasionally an entire tree is killed. More commonly, there is dieback of one-third to two-thirds of the top; then, a month or more after the initial wilting and death of leaves, new shoots appear from adventitious buds along the larger branches and the trunk, and vigorous new growth rapidly regenerates a new top. This renewal of growth coincides with, or closely follows, formation of new xylem tissue replacing that invaded by the fungus.

After the initial appearance of the disease, trees often completely recover and do not wilt again. Trees may wilt several years in succession, however. The disease has been observed in December, January, June, and July.

THE CAUSAL FUNGUS

Verticillium albo-atrum was first isolated from avocado trees at Vista, San Diego County, California, in December, 1947. This isolate and subsequent isolates from a number of other diseased trees were typical of *V. albo-atrum* as described by Reinke and Berthold and as clarified by Rudolph (4).[3]

Wilhelm (5) states that the conidial constituent of *V. albo-atrum* forms abundant black microsclerotia at temperatures of 10° to 22° C., while at 25° to 31° C. the colonies are creamy-white, with few microsclerotia. He further states that the stable white mycelial variants are unaffected by temperature. In the present experiments, the isolates from avocado showed the same responses to temperature as those noted by Wilhelm.

VARIETIES AND LOCATION OF AVOCADO TREES AFFECTED

Under natural conditions Verticillium wilt has been found on Guatemalan seedling trees (*Persea americana* Miller), on Guatemalan varieties (Anaheim and Queen) budded on Guatemalan rootstocks, and on the hybrid Fuerte variety budded on Guatemalan and Mexican (*P. americana* var. *drymifolia* (Schlechtendal and Chamisso) Blake) rootstocks. The rootstock types were determined by the method developed by Halma (2).

Various Mexican varieties comprise the majority of the rootstock sources for commercial avocado trees in California, although some Guatemalan seedlings have been used. The Fuerte is the principal variety under commercial production in California.

The disease has been found in all of the commercial avocado districts in California, including sections of San Diego, Orange, Los Angeles, Ventura, and Santa Barbara counties. Trees naturally infected have ranged from 2½ to 20 years of age. Since the disease usually occurs on only one or two isolated trees in a grove, it is not at present of economic importance.

INOCULATION

Avocado seedlings were inoculated in the greenhouse by two main methods: either by dipping roots in a suspension of spores and mycelium, or by injecting spores into the vascular system of the stem.

For inoculation by the first method, two 14-day-old Petri-dish cultures of *V. albo-atrum*, isolated from avocado trees and growing on potato-dextrose agar, were homogenized with water in a Waring Blendor. The roots of ten 10-month-old seedlings, five each of Mexican (Topa-Topa) and Guatemalan

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[3] The isolate from avocado was submitted for examination to Drs. B. A. Rudolph and S. Wilhelm of the University of California at San Jose and Berkeley, and was identified as *V. albo-atrum*.

(Anaheim), were dipped in the spore-mycelium suspension and planted in cans of sterilized greenhouse soil (Fig. 3), which were placed in temperature tanks, one each at 15°, 20°, 25°, 30°, and 35° C., in August, 1948. Tomato plants (Improved Pearson) were also inoculated by dipping the roots in the suspension and were planted in cans placed in the temperature tanks. Soil temperatures were maintained at 15°, 20°, 25°, 30°, and 35° C.

FIG. 3. Guatemalan (left) and Mexican (right) avocado seedlings inoculated by dipping roots in spore and mycelium suspension of *Verticillium albo-atrum*. Photographed 4 weeks after inoculation.

Twelve days after inoculation the first symptoms of Verticillium wilt appeared in three Guatemalan seedlings, one each at 20°, 25°, and 30° C., from all of which the pathogen was recovered. These seedlings wilted suddenly, and the leaves died within another week. Vascular discoloration was present in leaf petioles, and the fungus was recovered from the petioles. Disease appearance was delayed in this variety when grown at 15° C., and no symptoms appeared at 35° C.

The Mexican-type (Topa-Topa) seedlings were not so severely affected by the inoculation as the Guatemalan. Moderate wilting of the leaves oc-

curred in the tanks held at 20°, 25°, and 30° C., 3 to 4 weeks after inocula-
tion. Light vascular discoloration was present in the seedlings grown at
these three soil temperatures, and also in those grown at 15° and 35° C.
Plants grown at these two temperatures had no other symptoms of the dis-
ease, except a slight wilt of leaves at 35° C.

After the death and abscission of some of the leaves, new shoots appeared
on both types of seedlings grown at 20°, 25°, and 30° C., as under conditions
of natural infection. No symptoms developed on either Mexican or Guate-
malan control seedlings.

Inasmuch as a rather resistant variety of tomato was used, symptoms
were not obvious on these plants; there was some one-sided yellowing of
leaves, however, and *V. albo-atrum* was reisolated from the faintly dis-
colored portions of the petioles and stem.

Two Anaheim and two Topa-Topa seedlings were inoculated by the
second method, which has been used successfully in studies on the Dutch elm
disease and other vascular diseases (6). The stem of the avocado seedling
was placed in a horizontal position, a drop of concentrated spore suspension
was pipetted onto the stem, and the drop was cut through with a scalpel, the
cut penetrating into the xylem. The spore suspension is readily taken up
by the vessels in this method. Ten days later several leaves in the top of both
Anaheim seedlings and one Topa-Topa seedling showed a one-sided yellow-
ing and a drying-out of tissue. Vascular stain was heavy, and the fungus
was readily reisolated from leaf petioles in the top of the 18-in. seedlings.

PREVENTIVE MEASURES

The causal fungus in many cases does not survive in the invaded wood
of the avocado for any appreciable length of time. It is readily isolated
from branches at the time of or soon after the initial appearance of disease
symptoms, but a few months later it is often impossible to isolate the fungus,
even from heavily stained wood. Similar difficulty was noted in reisolation
from the artificially inoculated seedlings. This may be correlated with the
fact that many trees apparently recover completely from the disease. Re-
currence of the disease on some trees for several years in succession may
indicate a particularly susceptible rootstock. Such trees should be removed
from the grove, and if the area is to be replanted to avocado, the soil should
be fumigated with chloropicrin before replanting.

Obvious measures to reduce the possibility of disease occurrence include
not planting avocado trees on land that has been recently cropped to tomatoes
or other plants highly susceptible to *Verticillium albo-atrum*. Several
groves in which the disease has appeared were planted to tomatoes the year
before the avocado trees were set out. In the case of nursery plantings, the
use of land that has been in some other crop susceptible to *V. albo-atrum*
should be particularly avoided. Interplanting young groves with sus-
ceptible crops should also be avoided.

SUMMARY

The causal agent of a disease of avocado trees in California involving sudden wilting and collapse of the leaves has been identified as *Verticillium albo-atrum* Reink. and Berth. Trees usually are not killed entirely and often make excellent recovery.

The disease is present in all of the commercially producing avocado districts in southern California. Trees on both Mexican and Guatemalan rootstocks are affected. The disease had been found on both seedlings and budded trees.

Experimental seedlings inoculated by dipping the roots in a suspension of spores and mycelium showed disease symptoms in 12 days at soil temperatures of 20°, 25°, and 30° C. The Guatemalan seedlings were more severely affected by the inoculation than the Mexican.

Certain preventive measures are suggested.

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EFFECTS OF BACTERIUM TUMEFACIENS ON ALLIUM CEPA[1]

SOPHIE JAKOWSKA[2]

INTRODUCTION

Bacterium tumefaciens has been used experimentally not only to infect plants which normally succumb to it in their natural habitat but also to test the susceptibility of some plants which are ordinarily not infected by *B. tumefaciens*. The work of Smith and Quirk (36) on *Begonia,* which was immune from *B. tumefaciens,* gave the incentive to the study of other cases of unusual infection. *Allium cepa* was listed by these authors (36) as a plant unlikely to become infected because of the unfavorable hydrogen-ion concentration of the sap. Since no reports of *B. tumefaciens* infection of *A. cepa* had been made, it seemed desirable to test the susceptibility of this plant to *B. tumefaciens* with the hope of producing some form of crown gall on either the young seedling or on the adult bulb. There was also the hope that it might be possible to study such overgrowths from the viewpoints of general morphology and cytology.

The cytological effects of *Bacterium tumefaciens* infection were, if they existed, to be of considerable interest in view of the fact that cytological studies of the crown gall disease are restricted to the rather limited data from Smith, Brown, and McCulloch (35), Robinson and Walkden (33), Levine (21, 23, 24), Riker (30), Riker and Berge (31), and Winge (39). Winge's paper is the most extensive. His cytological information is not incidental but constitutes the subject of the investigation. Winge concluded that *B. tumefaciens* acts on living cells and that polyploid cells are found in galls caused by *B. tumefaciens.* However, reports of the actual presence of *B. tumefaciens* in living cells have been few and the evidence on the subject still is contradictory in view of the micrurgical studies of Hildebrand (11), who concluded that a single *B. tumefaciens* is unable to survive and reproduce after introduction into a living cell. More and more evidence is accumulating in favor of the view that auxin-like substances are produced by *B. tumefaciens,* and this theory would of course allow for the action of *B. tumefaciens* apart from its actual entrance into the living cell. It is still undetermined, however, whether the chromosomal constitution of cells involved in crown gall formation is an essential feature of infection by *B. tumefaciens.* In material susceptible to *B. tume-*

faciens, the study of abnormal mitosis with consequent changes in number of chromosomes failed to reach any degree of certainty, because size of chromosomes was the main limiting factor. For this reason, the attempt was made to infect with *B. tumefaciens* a plant whose normal cytology is well known, having large chromosomes which have been observed under a wide variety of experimental conditions.

The cytological approach to the problem of crown gall has been abandoned in most respects during recent years, but among the authors who have treated in detail cytological phenomena in plant tumors are Winge (39) in his work on the sugar-beet crown gall, Whitaker (37), who studied spontaneous tumors of *Nicotiana* hybrids, and Wipf (40), who investigated root nodules of Leguminosae. Their findings on macroscopically similar growth abnormalities differ greatly. Observing polyploidy in the majority of tumor cells in sugar-beet crown gall tissue, Winge supported the hypothesis that tumors result from somatic polyploidy. Wipf also noted polyploid cells in her study of root nodules of leguminous plants caused by *Rhizobium,* although some authors deny these root nodules a place among real plant tumors. Whitaker, however, did not find a single polyploid cell in his material. Mitosis was very regular in *Nicotiana* tumors and the few irregularities found were not considered significant. In normal stem tissues there were as many as four nucleoli within the nucleus. In tumor tissue most nuclei contained two nucleoli but their number varied within the limits for normal tissue. While these three tumors were macroscopically similar in certain respects, their cytological differences were great. Thus, although polyploidy or some other cellular phenomena were encountered in some cases, there seemed to be no common factor accountable for the formation of these overgrowths. It was hoped that in the present study it would be possible to produce some form of crown gall in which the cytological phenomena involving polyploidy might be accurately observed.

The problem of the study of plant overgrowths is complicated by the factor of specificity, or perhaps it should be expressed as a lack of specificity. It has been very commonly observed that *Allium cepa* bulbs which have been carelessly treated and mutilated by deep cuts in order to obtain a greater production of roots, manifest more or less irregularity in growth. With this in mind the author attempted to study briefly the specificity of action of *Bacterium tumefaciens* on *A. cepa* as compared with such agents as *Escherichia coli, Bacillus subtilis,* a synthetic hormone (diethylstilbestrol), and an auxin (indoleacetic acid), as well as chemical and mechanical injury. Among these agents, diethylstilbestrol has been reported by Lettre (17, 18) to inhibit mitosis in much the same manner as colchicine; it was also compared by Zollikofer (42) with plant hormones such as indoleacetic acid. It therefore seemed very desirable to study its effects on *A. cepa* since abnormal growth, often in the form of a tumor,

sometimes results from treatment with substances which possess a colchicine-like action.

MATERIALS AND METHODS

Small bulbs, as well as seeds, of *Allium cepa*, Yellow Globe variety, were used. Bulbs averaging 35 gm. in weight were selected for roundness and size of stem base; none were used which had an odd or irregular shape. All bulbs were grown in half-pint glass containers. Seeds were germinated in Petri dishes with one layer of filter paper 9 cm. in diameter. Generally some water was added to allow for evaporation except in those cases in which the experimental plants were kept in moist chambers. In some cases, roots from bulbs were treated in small containers of about 10 cc. capacity. Sterile instruments and containers were used wherever possible. All experiments were conducted in moderate light at approximately 24°–26° C. and in a few instances at 30° C.

Bacterium tumefaciens culture 4720 from Georgetown University was propagated by transfers on "Difco" dextrose agar. Onion decoction media, such as onion decoction and 2 per cent onion decoction agar, as described by Anderson (1) for a typical *Allium cepa* pathogen, *Urocystis cepulae*, were used either in their natural condition with the normal pH of 5.5 or titrated to pH 7.0. Cultures were grown at approximately 24°–26° C.

The effects produced by *Bacterium tumefaciens* were studied on growing roots from bulbs, on germinating seeds, young plants and bulbs, and in particular on the stems of bulbs. Roots from bulbs were grown directly in the bacterial culture on onion decoction medium or in tap water to which bacteria were added daily. Germinating seeds and young seedlings were brought in contact with the bacteria by introducing the bacteria into water used for moistening filter paper in the Petri dish. In other cases the epidermis of growing roots and young seedlings was scraped with a bacteria-laden needle; or an aqueous suspension of bacteria was introduced by means of a thin gauge needle into growing roots and young seedlings. Bulbs were inoculated with *B. tumefaciens* by various methods: particularly in the preliminary experiments, the injection of aqueous suspensions of bacteria in various regions of the bulb such as the growing green leaves, in and between the scales, and in the base of the stem; spreading of bacteria over a freshly scraped stem; growing the plants in the bacterial culture; pricking the center of the stem with a needle carrying bacteria; and transfers from previously produced infections. This last method was performed variously by applying infected tissue to stems of healthy plants, by injecting juice extracted from infected bulbs, and by reinoculation with bacterial cultures isolated from infected bulbs.

Bacteria were isolated from bulbs of *Allium cepa* in each case of infection. Gram stained smears were prepared and the general features of each isolated culture recorded for comparison with the original culture

used as inoculum. Media in which infected plants were grown were checked for the presence of *Bacterium tumefaciens*. The effects produced by the bacteria in respect to changes in hydrogen-ion concentration were studied in the case of infected plants and onion decoction media.

Various other agents were used, such as needle-prick inoculations of *Escherichia coli* and *Bacillus subtilis*, diethylstilbestrol in various concentrations ranging from 0.0025 per cent to 1 per cent solutions, a solution of 0.2 per cent indoleacetic acid, a solution of 1 per cent chloral hydrate, solutions of 0.5 per cent and 0.25 per cent podophyllin, and an injection of 0.025 cc. of glacial acetic acid. The effects of diethylstilbestrol (Merck & Co.) were studied on the germination and the development of seedlings, on roots growing from bulbs, and on excised root tips as well as on the stems of fully grown bulbs. These results were reported elsewhere (14). Deep cuts by which most of the stem was removed from the bulb were made to study the effects of mutilation. Treated and control material was examined cytologically at frequent intervals.

The cytological technique consisted of the following: fixation with 1:3 acetic-alcohol for smears and with Craf for sections; staining with Feulgen with or without fast green counterstain and with aceto-orcein for smears; iodine gentian violet, Feulgen, and iron-alum haematoxylin for sections cut at 10 micra. Gram stain was used for bacteriological smears.

MACROSCOPICAL AND BACTERIOLOGICAL OBSERVATIONS ON MATERIAL TREATED WITH BACTERIUM TUMEFACIENS

Roots from bulbs growing in tap water to which *Bacterium tumefaciens* was added daily showed no external effects of the treatment during one week. A yellowish discoloration, particularly in the root cap region, of some of the roots grown directly in cultures of the bacteria in an onion decoction at pH 5.5 or pH 7.0, with clear evidence of wilting in a few cases, was most probably not caused by the bacteria because the sterile media produced similar effects. Attempts to introduce bacteria into the roots by scraping roots with a needle or injecting minute amounts of an aqueous bacterial suspension resulted in only occasional side roots at or near the point of injury. It was impossible to infect bulbs by needle prick of the roots or by placing the roots in a bacterial suspension.

The treatment of seeds with water suspensions of bacteria did not impair their power of germination. Seedlings grown in the presence of bacteria had no visible signs of infection and all attempts to produce such signs by pricking the young seedling either before or after the appearance of chlorophyll were unsuccessful. The young seedlings treated with *Bacterium tumefaciens* were not raised to maturity and thus it is impossible to establish whether this stage in the life of the onion plant is in reality immune from *B. tumefaciens*. Izrailskij (13) obtained 20–30 per cent infection by treating seeds of *Beta vulgaris* with a culture of *B. tume-*

faciens. It may, however, be supposed that the bacteria responsible for this 20-30 per cent infection did not actually infect the germinating seeds but, by surviving near the seedlings, infected them later whenever a wound provided a portal of entry. This seems plausible in view of Izrailskij's report that 100 per cent infection followed the pricking of young plants, with consequent introduction of bacteria.

Various methods of inoculation were employed in preliminary experiments to ascertain the susceptibility of *Allium cepa* bulbs to *Bacterium tumefaciens.* A true case of infection generally followed inoculation involving the base of the stem. However, the time necessary for manifestation of symptoms was variable. The most successful method of inoculation, employed throughout the experiments as the principal means of infection, was by needle prick into the center of the stem base. No visible effects were obtained when the bacteria were introduced into the upper portion of the bulb, which consists of scaly leaves only. Needle-prick inoculation has produced infection in more than 30 bulbs (Figs. 1-3). However, an approximately equal number of bulbs did not have the expected symptoms of infection after needle-prick inoculation. This could be explained by changes in virulence of *B. tumefaciens* cultures. Such changes were reported by early investigators and confirmed by Levine (25) and others.

The first appearance and progress of the infection was easily observed. As early as two days after needle-prick inoculation, a slight swelling could be detected at the base of the bulb just above the margin of the scale leaves. Some control bulbs also had a slight swelling detectable only by palpation, but this swelling never developed further into a localized abnormal overgrowth as in the case of bulbs infected by *Bacterium tumefaciens.* The bulbs remained apparently healthy except for the widening of the stem base along one diameter and a loosening of the external scales during the early stage of infection, usually within a week after inoculation. In some cases the widening of the stem was accompanied by a local break in several layers of scales and an enlargement of the root-bearing area.

A typical abnormality which usually developed in about 13 days, or in some plants 8 days, consisted of a horizontal elongation of the stem base. The outer scales at one end of this elongation were broken and a white bulbous swelling, suggesting a kind of tumor, appeared from beneath. This tumor or overgrowth was composed of the inner scales which manifest a degree of curvature different from that of the other scales. The loss of symmetry was very evident. The base of the stem in the controls maintained its circular shape and reached the approximate size of 16 mm. × 16 mm., compared to 20 mm. × 16 mm. in infected plants.

This outpushing of inner scales occurred gradually and after reaching a definite size, within approximately 2 weeks, did not progress further. From this time on degenerative changes took place in the outer portion of
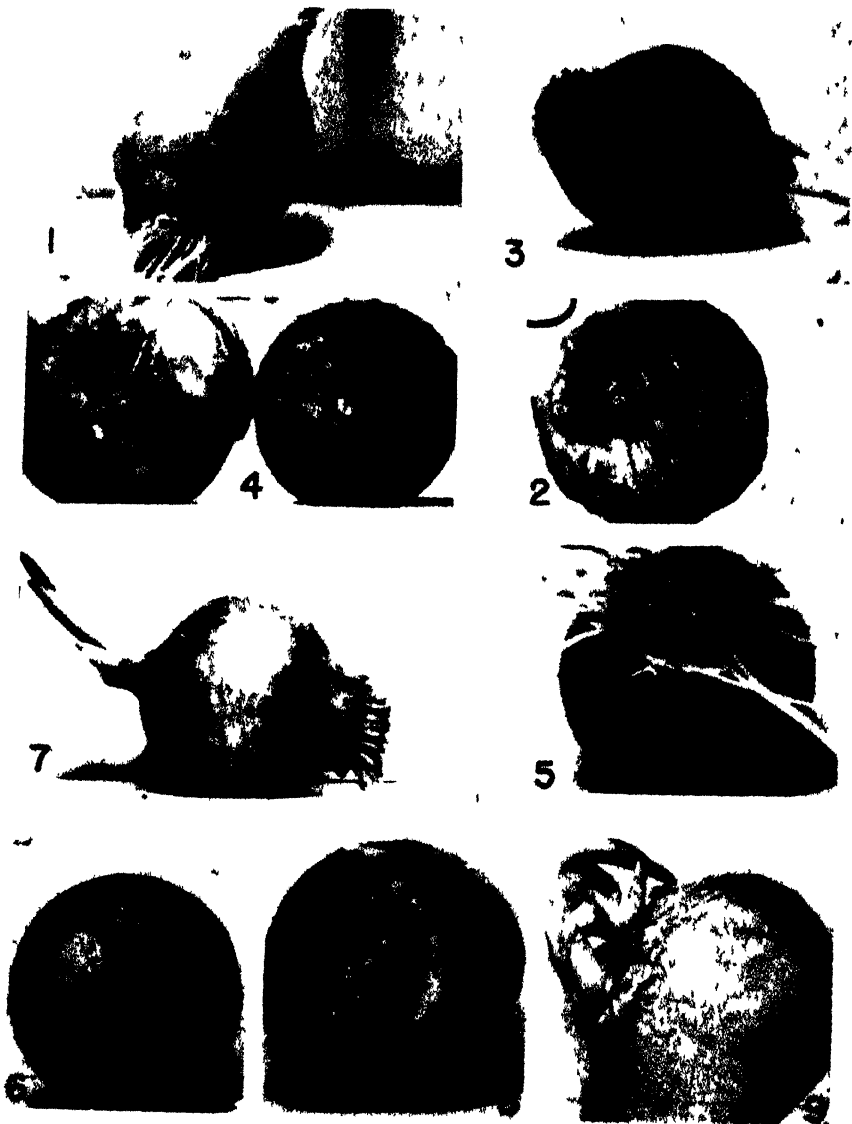
FIGS. 1 to 9. Bulbs of *Allium cepa* about ⅔ normal size.

(1) Seven days after needle prick inoculation with *Bacterium tumefaciens*. Widening of stem base and loosening of outer scales.

(2) Twenty-two days after needle prick inoculation. Spreading of stem, bulbous projection at one side of stem, and advancing degeneration of tissues. Roots have been removed.

(3) One month after needle prick inoculation. Roots have been removed and plant is somewhat dried. Center of stem shows area injured by inoculation.

(4) Application of tissue from a diseased plant to a healthy bulb results within 14 days in destruction of tissue in the new host.

(5) Spreading of stem 18 days after application of tissue from a diseased plant.

(6) Plant treated for 13 days with 1 per cent solution of chloral hydrate after 24 hr. recovery in tap water. Roots and leaves suppressed; base of bulb slightly swollen.

(7) Plant treated with 0.2 per cent indoleacetic acid in the form of lanolin paste applied to needle-prick wound in center of stem. Stem vertically elongated.

(8) Injection of 0.025 ml. of glacial acetic acid in center of stem base resulted within 11 days in severe injury with necrosis spreading in all directions.

(9) A deep cut in the base of the stem caused abnormal growth of leaves downwards through cut surface.

the bulb, involving the stem and the lower portion of the scales. Frequently the center of the stem showed injured regions where the bulbs were inoculated. After the roots had been cut at this stage no new roots appeared, because of the degeneration of root primordia.

The inoculation of *Allium cepa* bulbs with *Bacterium tumefaciens* resulted in a disease which caused premature death of the plant. Before decay was complete, the bulbs showed evident signs of wilting which could be attributed to the fact that all the vessels supplying the upper portion of the leaves had been destroyed together with the other tissues. Dying plants often were attacked by molds such as *Rhizopus* and a pink mold, probably *Neurospora*. The described effects were never obtained after similar inoculation on plants growing in water at 30° C. Eradication of the diseased area even as late as the 20th day after inoculation preserved the life of the plant. This seemed to indicate that the bacteria were limited in most cases to the regions adjacent to the point of inoculation injury and, moreover, the bacteria were concentrated near the necrotic tissues at the surface. This would be in accord with the aerobic habit of *B. tumefaciens* and its normal behavior in the host, as noticed by early investigators (33). Plating out of the tissues from diseased plants yielded colonies of *B. tumefaciens* with milky-white coloring, gumlike consistency, and entire borders on dextrose agar. Similar cultures were obtained when the fluid from the lower part of the diseased bulb was drawn out with a sterile syringe and plated.

The production of infection in healthy bulbs by means of tissue taken from infected bulbs was successful in 15 instances. Identical characteristics of the original disease were produced by direct application of a piece of diseased tissue to a healthy plant (Figs. 4, 5), by injection of sap of the diseased plant, and by use of cultures isolated from infected plants. By this last procedure in which *Bacterium tumefaciens* isolated from an infected onion bulb was used to produce a new infection, the etiological agent was ascertained according to Koch's postulates. From all these cases of infection a Gram negative bacillus, similar to that of the original culture, has been isolated. In some instances the shape of the bacteria isolated from the plant differed slightly from the original culture, being shorter and sporelike in appearance, but, on culturing in agar medium, the bacterium recovered its original characteristics. This observation seems to be another instance of the morphological changes in *B. tumefaciens* which have been pointed out by early investigators such as Levine (22, 25).

The effects of infection by bringing a piece of diseased tissue in contact with a healthy stem were seen as a widening of the stem base and a bulging which caused breaking of the outer scale covering. Fourteen days after application, degeneration of the tissues was apparent. It seems that the bacteria passed from the diseased tissue to the healthy tissues of the stem, which represented a more adequate substrate. Their access was

facilitated by the fact that in scraping off the old roots some regions were inevitably injured. The crushed cells from this small area or areas of injury provided an entrance into the new host, while at the same time the bacteria were harbored in relatively large numbers very close to the stem in the infected necrotic tissue that had been applied to it.

All of these cases of infection by *Bacterium tumefaciens* seemed to indicate that *Allium cepa* was susceptible to this form of crown-gall disease when fully grown bulbs were raised in tap water. This appeared to be contrary to the expectation of Smith and Quirk (36) based on the pH range which was believed to be tolerated by *B. tumefaciens*, although Berridge (3) considered pH 5.2 in the plant sap as most favorable to growth of *B. tumefaciens*. The case of *A. cepa* is not an isolated one, since many of the plants supposed by Smith to be naturally immune from *B. tumefaciens* have been proven susceptible to this organism when it is artificially inoculated.

The pH of *Allium cepa* sap as measured by the author was between 5.0 and 5.5. The pH of the sap in infected plants was between 6.5 and 7.0. This was in agreement with the findings of other investigators who also observed that galls produced by *Bacterium tumefaciens* were more alkaline than normal tissue of the host plant (7, 15, 16). Thus the pH of the onion juice did not noticeably inhibit in any way the action of the bacteria, especially when the bacteria were placed on a tissue which was in part bruised by the inoculation. In experiments with *B. tumefaciens in vitro*, it was found that the bacteria had the ability to change the pH of onion decoction medium from 5.5 to 6.5 in 48 hr. at room temperature (24°–26° C.), while the pH of the sterile decoction remained 5.5.

The effects of *Bacterium tumefaciens* on *Allium cepa* bulbs are not similar to what is commonly known as crown gall. It was not a wartlike growth with a rather dry covering; it did not resemble closely the other types of crown gall such as the leafy crown gall, the wooly knot type, and the fasciation type. Most naturally occurring crown galls depend on the vessels of the plant for water, but this supply is limited when constriction occurs at the point of junction between the gall and the stem. Such a gall has its period of growth, development, and senescence, as well as death, and all these can occur independently of the host (21). However, the water supply of the onion overgrowths, composed of scales and stem base, was practically unlimited since the bulbs were partially immersed in water. Moreover, in the *A. cepa* overgrowths, the infected portion was an integral part of the plant and the fate of the overgrowth and the infected bulb was identical.

Dried infected bulbs kept at 100° C. for several hours a day for more than 20 days were used to inoculate other healthy plants, but no effects were produced by the application of such dried diseased tissue. Evidently the bacteria which were the agents responsible for the described effects were completely destroyed by heat. The thermal death point of *Bacterium*

tumefaciens was described in the literature as 50° to 51° C. for 10 minutes. Even lower temperatures of 46° and 47° were considered injurious when applied for prolonged periods (4).

The bacteriological observations seemed to definitely attest to the presence of *Bacterium tumefaciens* in the infected plants. Short Gram negative rods similar to those of the original culture of *B. tumefaciens* have been isolated in each case of infection. The stem surface plating of the infected plant on agar media revealed the presence of Gram negative bacteria believed to be *B. tumefaciens,* as well as some longer and more slender Gram positive rods similar to those found in tap water. The fluid drawn out of the infected plants near the stem base contained cell debris and Gram negative bacteria, as well as the mentioned Gram positive rods. The tissue of dying malformations on the infected plants about 38 days after inoculation, smeared by ordinary bacteriological methods, showed Gram negative rods among the cell debris. Sap drawn from the portion of the bulb above the stem and between the scales contained Gram negative bacteria. Numerous Gram negative bacteria were obtained from the water in which an infected plant had been growing for 36 days. Thus, by bacteriological methods it could be established that the bacteria inhabit the decaying tissues and are found at the surface rather than within the diseased stem. This surface position renders it an easy matter for the bacteria to fall into the surrounding water whenever the decaying tissue breaks away. The bacteria also are found in the sap above the stem and in the stem portion that becomes rather soft as infection progresses. *B. tumefaciens* was not observed, however, either in Feulgen smears of the infected tissue or in paraffin sections stained with either iodine gentian violet or Feulgen. Many authors, among whom are Robinson and Walkden (33), were unable to see the bacteria in the tissues although they could ascertain their presence by plating out the diseased tissues. Whenever the bacteria were seen within the cells, which was reported as only occasional by Riker (29) and Magrou (27), it was doubtful whether the small bodies seen within the cells were the bacteria or some cytoplasmic constituents of the cells. For this reason, Milovidov (28) proposed a rather complicated procedure for the differential staining of bacteria and mitochondria, while Wright and Skoric (41) suggested the use of Giemsa stain with a pre-treatment with a buffer solution. The latter methods are of great value in the study of root nodule bacteria in Leguminosae but were not employed in this investigation. More recently, Brown and Evans (6) claim to have observed the bacteria in cortical cells of the roots, in tracheids, and in medullary ray fibers of conifers infected by *B. tumefaciens,* but they do not mention the method used. In *Allium cepa* the only indication of bacteria are strands of darkly staining tissue, apparently necrotic, which infiltrate among the normal tissues and seem to be identical with what Robinson and Walkden (33) referred to as "zoogleal masses."

MICROSCOPICAL OBSERVATIONS ON MATERIAL TREATED
WITH BACTERIUM TUMEFACIENS

Detailed observations of roots and seedlings treated with *Bacterium tumefaciens* yielded no result of cytological importance. Evidently the bacteria never penetrated the roots, and no sign of injury could be detected. Even in roots and seedlings which had been pricked, there was only limited development of side roots. The regions in which the side roots arose showed, as was expected, a large number of perfectly normal mitotic divisions. It may be concluded that the presence of bacteria did not disturb the development of roots and seedlings.

Roots growing from bulbs in the onion decoction (at pH 5.5 or 7.0) which had been heavily inoculated with *Bacterium tumefaciens* were not significantly affected by the bacteria. The number of mitotic divisions in the meristem appeared normal, with all stages of mitosis present. The same material was studied in sections since it was necessary to make certain whether or not any effects were produced on the regions of elongation. This effect would be expected if some auxin-like action were involved, since it was demonstrated (2) that divisions may be obtained in the region of elongation by the application of naphthalene acetic acid. However, divisions were not observed in either the region of elongation or region of differentiation. Aceto-orcein smears were made daily throughout one week in order to study the cells of stems taken from bulbs which had been inoculated by needle prick, but they did not yield significant observations.

The best preparations for the study of position and relationship of cells were sections cut at 10 micra and stained with iodine gentian violet. Six days after the inoculation, the normal morphology of the stem was in no way altered except for the point of inoculation. It was possible to distinguish the definite pith and cortical parenchyma as well as numerous root primordia at the periphery of the stem. The vascular bundles appeared rather regularly scattered throughout the cortical and pith parenchyma. In close proximity of the needle-prick inoculation point, a region of cells which stained deeply, the cell walls were colored a deep blue with gentian violet. These cells were often devoid of nuclei, or some nuclei appeared notched and crenated and simulated some of the so-called amitotic fission stages to which early investigators (35) attached great importance. These features, however, were not characteristic inasmuch as they generally can be found in most injured tissue. Of course, their number in this early stage was very limited but increased as the infection progressed.

When bulbs were grown directly in the bacterial culture rather than in tap water, it was possible to notice a path of destruction very probably followed by bacteria, although the bacteria could not be demonstrated by staining. The portions between the root primordia seemed to become involved in the early stages, and destruction of the tissue proceeded toward the vessels. The destroyed tissues appeared to block the usual path of the

flow of water to the upper regions of the plant. Intercellular spaces left by the collapsed dead cells were also observed at this stage. Above the root-bearing area, the region between the basal portions of the scales was subject to destruction by the bacteria or by the products of the bacterial culture, particularly the cells along the margin of such scales, and the parenchyma cells. The effects of inoculation by needle prick, however, were more limited and localized. Even 28 days after inoculation the periphery of the stem remained fairly healthy, while the region around the point of inoculation was destroyed. The destructive effects were more evident in the outer scales in which at this time growth stopped and a slow wilt and decay began.

The amount of decaying tissue in the stem proper varied from plant to plant. Thus the sequence of the histological features discussed below does not necessarily represent the sequence of events during *Bacterium tumefaciens* infection. The sequence was as follows: 6-day-old cross section, 10-day-old longitudinal section, 15-day-old cross section, 19-day-old cross section, 21-day-old cross section, 28-day-old cross section, and 32-day-old longitudinal section. It is important to remember that the response to treatment of the same set of experimental plants occurs at varying times; thus the typical overgrowth composed of a spread stem and excessive curvature of scales occurred on the average after 13 days, but in some plants after 8, 12, 14, and 19 days. The material for microscopical observations was chosen according to the general macroscopical response which was common to most bulbs at definite times. In the early stages of the disease it was easy to preserve entire stems with several attached scales, but as the disease progressed it was frequently impossible to fix the disintegrating scales. Hence, in the later stages it was necessary to use material from the stem alone and in particular from the portion nearest the overgrowth. It was believed that this portion would reflect the true condition of the plant Few general statements can be made in regard to this material. True disintegration of tissues involving large areas did not appear before the 15th day after inoculation (Fig. 13). There were no divisions in the stem tissue although this tissue clearly was spreading. Divisions were seen occasionally in leaf and root meristems and in pericycle cells in the differentiated regions of roots. There were many more cells in 15-day material than in 6-day material. Cells were small but no division in the 15-day material could be seen. In general, none of the material except that treated for 10 days with bacterial culture had dividing cells. This lack was understandable in the later stages when a general degeneration of tissues set in, but some dividing cells might have been expected during the early stages when size of stem and of basal portion of scales was increasing. Several factors, however, can also be considered responsible for the early macroscopical changes, such as the increase in the root-bearing area due to observed activity of the pericycle cells, and the formation of intercellular spaces which by passive intake of water may increase the size of the bulb.

FIGS. 10 to 12. Effects of a 10-day treatment of *Allium cepa* bulbs in a liquid culture of *Bacterium tumefaciens*. Longitudinal sections, stained with Iodine-Gentian Violet.

(10) Upper portion of stem and basal portions of scales. A polyploid cell was observed in basal region of central leaf meristem which stands out as the involuted structure in center of field. (×24)

(11) Dividing cell in cortical parenchyma of stem below central leaf meristem. (×768)

(12) Polyploid cell from region shown in figure 10, containing tetrachromosomes. (×1568)

Divisions first were observed in the cortical parenchyma in 10-day-old material which had been treated with bacterial culture (Figs. 10–12). In the cortex of leaves there were definitely elongated cells which contained very small amounts of cytoplasm but were in division. The most striking feature of this tissue, however, was the variable size of nuclei and chromosomes in adjacent cells of approximately the same dimensions. There was some indication of polyploidy, although an accurate chromosomal count was extremely difficult. A typical polyploid cell with some tetrachromosomes was observed in another region among cortical cells near the basal region of the central leaf meristem (Fig. 12). The tips of the tetrachromosomes showed a characteristic separation which indicated their doubleness. The size of the chromosomes and of the metaphase plate were definitely greater than that of ordinary diploid cells. A careful count gave 28 + chromosomes. The occurrence of polyploid cells in this material has not been frequent enough to indicate that polyploidy is an essential feature of *Bacterium tumefaciens* infection. It was impossible to establish polyploidy on the basis of number of nucleoli, since most of the cortical cells having very large nuclei contained two rather large or even a single unusually large nucleolus. The occurrence of polyploid cells in division, limited apparently to this material which had been treated with *B. tumefaciens* culture for 10 days, was the only instance in this investigation in which polyploidy could be definitely established by chromosomal count. In the normal material grown in tap water no such polyploid divisions were observed, but many cells of the cortical parenchyma appeared as large as the polyploid cells actually found in the treated material. This treated material had been grown in an onion decoction medium in which *B. tumefaciens* was growing and to which the bacteria added their metabolic products during 10 days. This combination of factors may have had a stimulating effect on the polypoid cells and thus revealed their nature, since a sterile onion decoction alone did not produce this effect. The same is true of *B. tumefaciens* when applied alone by needle-prick inoculation. Thus, if the divisions obtained in this case were to be considered as effects of the combination of factors, it should be suggested that this combination of factors did not produce but helped to reveal pre-existing polyploidy. It is possible that in the adult onion bulb great numbers of cells in the cortex are in the polyploid condition but do not undergo division.

Older stages of the disease were studied first since it was necessary to ascertain the outcome of inoculations. The macroscopical observations were carried on in some cases until the plant died; in others, the plant was fixed after the appearance of the most drastic symptoms of disease, such as general wilting and beginnings of decay, but in time to obtain tissue which could withstand handling. In the study of the earlier stages the early symptoms were noticed and it was inferred that the inoculation had resulted in infection and the plant could be expected to develop other symptoms later. It was of great disadvantage that material from the same plant

could not be collected at various time intervals. Excision of the infected
stem tissue effected a cure of the plant, especially when the bacteria were
localized near the point of inoculation. Thus the considerations and com-
parisons between the material of 2 and 6 days after inoculation are not
significant, since they refer to material that was taken from different bulbs,
although the bulbs were undergoing identical treatment. However, when
longer time intervals were allowed for greater changes to take place, the
results could be considered as roughly representative of stages of the dis-
ease.

When 6-day-old material was compared with 15-day-old material, the
most striking feature in the older material was a disorganization of all tis-
sues (Figs. 13–15). The decay manifested itself mostly at the periphery
of the base of the bulb in the regions occupied ordinarily by the bases of
the scales. The stem proper in cross section revealed a destruction of root
primordia, the most outstanding feature being a strandlike formation of
parenchyma cells, very numerous and compact, which appeared to infiltrate
the stem periphery.

As the degeneration of tissues advanced, more and more intercellular
spaces were formed. Root centers were definitely destroyed. Some nuclei
were highly pycnotic, others vacuolated. No mitotic divisions were present.
Many parenchyma cells were devoid of nuclei and cytoplasm. Frequently
cells of the vascular bundles had notched and crescent-shaped nuclei. Ex-
tranuclear chromatic granules were numerous. Formation of such nuclear
fragments in the cytoplasm has been observed by many authors on crown
gall material. Related phenomena characteristic of cellular and nuclear
degeneration have likewise been reported. In all these advanced stages
the vascular bundles were less numerous than in healthy stems. The great
relative increase in cortical parenchyma cells may be a source of error in
estimating the number of vascular bundles. It may be that the formation
of new bundles, as it takes place in a monocotyledonous stem, may have
been prevented in some way by the infection.

The main features accompanying the infection by *Bacterium tume-
faciens* as seen in these microscopical preparations can be related to the
macroscopical progress of the infection. The large increase in the number
of cortical parenchyma cells, so characteristic in 15-day preparations and
verified by the divisions observed in 10-day treated material, roughly coin-
cides with the period of development of the abnormal growths on the bulb.
The increase in the root-bearing area is correlated with the divisions occa-
sionally seen in pericycle cells of the differentiated regions of the root which
lead to the formation of new roots. The formation of intercellular spaces
in the early stages of infection by destruction of cells and the consequent
imbibition of water seem to be related to the bulging of the scales. The
final necrosis of a large part of the stem with the eventual wilting and
death of the plant is undoubtedly related to the destruction of vascular
elements of the stem and destruction of the cells which become devoid of
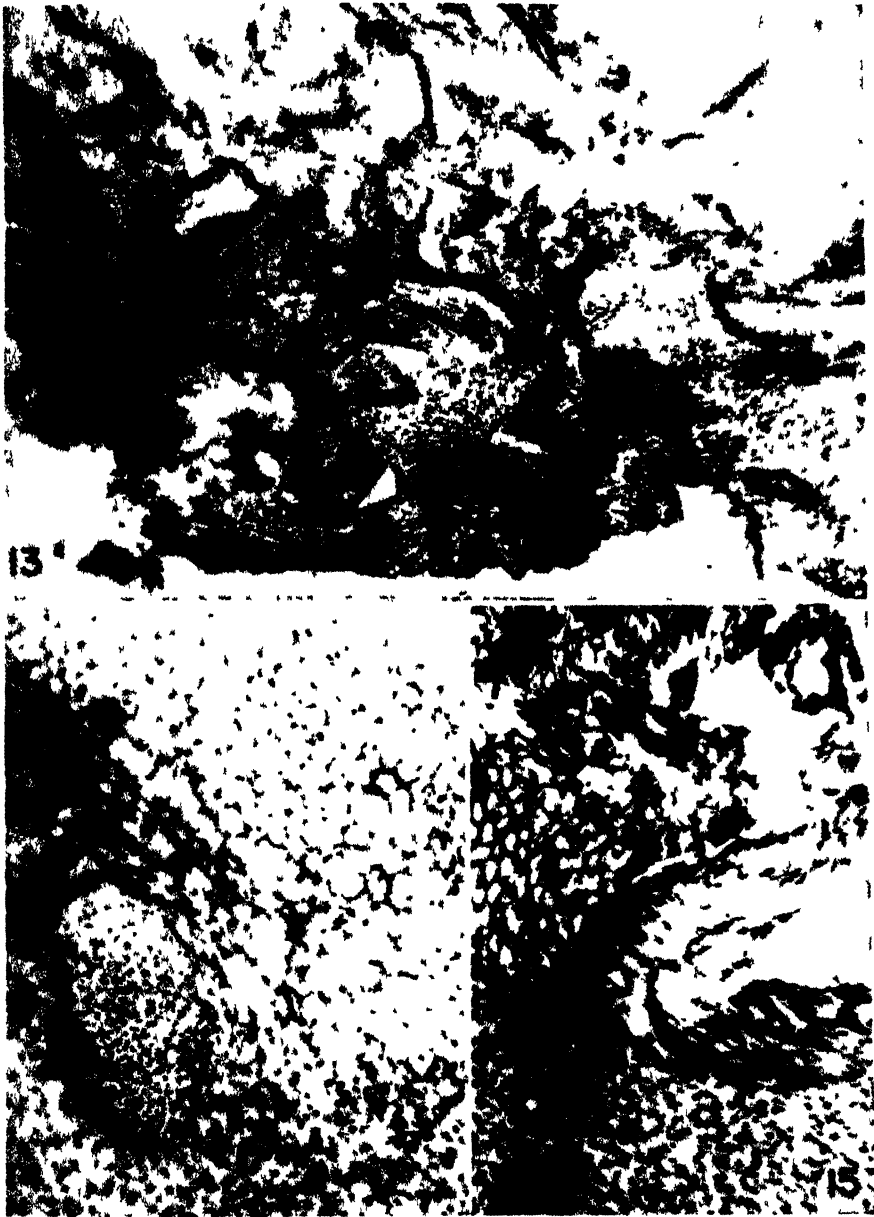
Figs. 13 to 15.   Late stages of infection produced on *Allium cepa* by needle-prick inoculation with *Bacterium tumefaciens*

(13) Destructive changes observed in cross-section of stem 15 days after needle-prick inoculation.   Iodine Gentian Violet.   (×24)

(14) Areas of pycnotic nuclei and of dead parenchyma cells near a degenerated root primordium in the same plant.   (×80)

(15) Another region in the same plant showing advanced degeneration of tissue and numerous intercellular spaces.   The root primordium is completely destroyed. (×80)

nuclei and cytoplasm and finally collapse. Polyploidy as seen in 10-day treated material is limited to the cortex near the leaf meristem and possibly the leaves, but it is not a pronounced feature of the infection.

<div align="center">DISCUSSION</div>

Previous studies of crown gall have been made almost exclusively on dicotyledonous plants. The infection of *Allium cepa* by *Bacterium tumefaciens* is in this respect unusual. The bulbs raised in tap water in the laboratory appeared actually infected and not merely carriers of the bacteria which were later isolated from them. No infection was produced by *Escherichia coli* or *Bacillus subtilis*, both of which are nonpathogenic to plant and animal. The effects produced by *B. tumefaciens* were characteristic in their gross appearance and could not be considered identical with those produced by other agents, although it is not excluded that other agents which would have provided the same type and amount of stimulation as did the bacteria might have produced similar effects.

Crown gall is ordinarily a disease of the stem but other parts of the plant may be attacked. The stem in *Allium cepa*, the part which responds promptly and visibly to the bacterial infection, differs greatly from the usually erect stem of a flowering plant. It is very limited in size and it is overshadowed by the large mass of fleshy scale leaves as well as by the large number of adventitious roots which arise from the base of the bulb. The stem of the onion bulb which was inoculated in these experiments was immersed in water. The resulting effects, which differed in a way from the commonly known crown gall, could be attributed to the morphology of the *A. cepa* bulb and to the method of growing the inoculated bulbs.

The response of *Allium cepa* to *Bacterium tumefaciens* at first sight appeared atypical, but it is important to remember that the essential feature of crown gall disease is the causal relation of some macroscopic localized manifestations of disturbed growth with the presence of *B. tumefaciens*. It is well known that crown gall may take a variety of forms. It may be covered or naked, irregular or regular, soft or hard. The gall is not necessarily on the crown of the plant; it may even be on the roots. The gall may readily decay, especially on soft plants, and wilting is one of the accompanying features (5). A wilt-inducing factor has been recently isolated from *B. tumefaciens* (12). On the other hand there are many galls on plants which can easily be mistaken for crown galls, such as knots or galls of the olive tree which are caused by *Phytomonas savastanoi*, nematode galls on yellow flax, and wooly galls due to aphids. Oleander galls caused by *Phytomonas tonelliana* are also very similar to crown galls. All these galls, while they resemble crown gall, lack the essential characteristic of the causal relationship to the presence of *B. tumefaciens*.

The overgrowth in *Allium cepa* resulting from inoculation with *Bacterium tumefaciens* was a localized disturbance involving the lower portion of the scales and the base of the stem and was caused by the *B. tumefaciens*.

Sterile needle prick experiments did not produce overgrowths of any kind. In addition, no results were observed when diseased tissue, which had been dried by heat, was applied to healthy plants. Finally, the plant could be restored to health when the regions near the point of inoculation were eradicated at an early stage of the infection.

The infection produced on *Allium cepa* bulbs by *Bacterium tumefaciens* had definite constant features. There was a proliferation of the stem in a horizontal direction and an increase in the root-bearing area, although *B. tumefaciens* did not act directly on the healthy roots. There also was a definite curvature of the leaves at their base in a limited region of the bulb. The pH of the sap of infected plants was less acid than that of the normal plant grown in water for the same length of time. Decay was a characteristic feature.

Bacterium tumefaciens in *Allium cepa* was primarily destructive in its effects and it was possible that the abnormal growth of stem and leaves was a secondary effect. This abnormal growth could be considered as a response to bacterial stimulus but it could also be a defensive reaction by which the plant attempts to develop away from the point of injury. This could be equally true of chemical and physical agents. The parallel between the effects of bacterial stimulation and of a strong acid, glacial acetic, is somewhat striking. The acid was applied in minute quantities to the stem by injection and not by needle-prick inoculation. The subsequent spreading of the stem away from the point of injury in the latter case was similar to the reaction in the case of infection by *B. tumefaciens* (Fig. 8). *B. tumefaciens* was able, however, to produce not only a response which could be interpreted as a defensive reaction, but also a typical growth response expressed in curvature of the leaves.

The histological observations suggested that the bacteria were extracellular rather than intracellular. By their presence the bacteria apparently caused a higher concentration of substances outside the cells and thus produced an osmotic flow from the cell interior which resulted in collapse of the cell walls. If the bacteria were within the cells and there elaborated products of their metabolism, the opposite situation may have occurred. Yet the characteristic feature of older stages of infection is the presence of collapsed and compressed cells. From micrurgical studies of Hildebrand (11) there is some evidence in favor of the idea that the bacteria causing crown gall do not live within the living cells.

Although the microscopical and macroscopical observations were definitely correlated, it was still a point of discussion whether there was correlation between the bacterial action and chromosomal phenomena, and whether the bacterial action could be considered colchicine-like, thus leading to polyploidy, or auxin-like, thus stimulating divisions in the region of elongation. Havas (10) considered the action of *Bacterium tumefaciens* as colchicine-like. While it was impossible to find any results which would indicate that *B. tumefaciens* was able to influence cells in division by inhibi-

tion of spindle, even by using heavy bacterial suspensions of *B. tumefaciens* on growing root tips, there seemed to be an indication that *B. tumefaciens* produced some kind of growth response with consequent stimulation of cell divisions in the elongated and extremely vacuolated cells. It also appeared that there was a stimulation of divisions of the polyploid cells of the cortex since these cells are not observed to divide in normal material. There is, however, controversial evidence as to the auxin content of crown gall tissues as well as to the amount of beta-indole-acetic acid produced by *B. tumefaciens in vitro* as compared with the quantities necessary for the production of overgrowths similar to those produced on plants by *B. tumefaciens*. Link and Eggers (26), who found that more auxin is present in hypocotyls inoculated with *B. tumefaciens* than in healthy hypocotyls, believed that disturbance in auxin relationships in infected plants played a role in gall development. On the other hand, Riker, Henry, and Duggar (32) could find no significant difference in auxin content of tissues treated by *B. tumefaciens* and the untreated tissues, and they concluded that other factors in addition to auxin were responsible for overgrowths and in particular for the characteristic pathogenicity of *B. tumefaciens*. The mechanism of plant overgrowth production by *B. tumefaciens* still remains undetermined.

The cytological observations on tissue infected with *Bacterium tumefaciens* were also inconclusive. Physical or chemical agents that could be applied to a plant in "pure" form helped eliminate many unknown factors, but the crown gall bacteria as living systems introduced many unknown factors. At the same time it should be noted that no well-defined and certain cytological effects have been reported in the literature. The authors who studied crown gall cellular phenomena often agreed in their description of the degenerative changes such as the breakdown of nuclei, notched and cleft nuclei, as well as furrowed nuclei simulating amitosis. These authors also referred to occasional binucleate cells and to various cell inclusions which they attributed in most cases to the failure of cell wall formation. There seems, however, to be no agreement concerning the role of the chromosomes in the crown gall disease. Riker (30) did not obtain evidence for amitosis, which was one of the favored theories of Smith (34), nor did Riker obtain evidence for multinucleate cells in crown gall tomato tissue. He believed that in consequence of repeated cell divisions nuclear size was reduced. On the other hand, Levine (21) observed in *Beta vulgaris maritima* giant cells with 4n and 8n chromosomal complexes, and attributed the production of higher chromosome numbers to the failure of chromosomes to separate as well as to the failure of cytokinesis ·to follow karyokinesis. Winge (39) is the investigator who attached utmost importance to the occurrence of polyploidy in sugar-beet tumors produced by *B. tumefaciens*. Winge determined that the majority of cells were polyploid, not only by chromosomal counts but also by the difference in diameters of cell nuclei. From his description of cells found in beet tumors, it seems that Winge

was possibly dealing with a case of polysomaty similar to that found in *Spinacia* (9). The so-called "diakinesis-like" somatic metaphases would indicate the paired metaphase plates as found in the first division immediately following double reduplication in the resting stage. The assertion by Winge that at the time he was entirely familiar with the normal cytology of *Beta vulgaris maritima* and that only diploid cells were found in the root tips studied, could not withstand more recent evidence. Levan (20) reports that in second-year root tips in sugar beets there is normally a chromosomal doubling. The tumors studied by Winge arose during the winter and it can be supposed that in the time required several reduplications of chromosomes in the resting stage may have occurred. Winge was convinced that the polyploid divisions he found in gall tissues were due to the *B. tumefaciens* infection. He was impressed by the mitotic disturbances leading to changes in chromosome number in animal tumor tissues and by the idea that the chromosomal disturbances might be the cause of malignant tumors in general. The frequency of polyploidy in the beet tumors seemed to substantiate his opinion, especially since he was convinced that normal cells in the root tips contained only diploid numbers of chromosomes. Although Levan's results were based on a small number of roots and are probably not sufficient to establish conclusively that polyploidy is in this species a part of the normal development, they indicate that polyploid tissue may occur in sugar beets over a year old. It is possible that in the case of crown gall on beets polyploidy was not caused, but was revealed, by the infection and by the growth-stimulating effects of *B. tumefaciens*, especially in view of the fact that age and cold storage are known to contribute to chromosomal doubling.

Most authors did not refer to meristematic tissues. Even when tissue was described as embryonic, it actually appeared as parenchymatous. The bacteria were localized in either xylem vessels or within dead cells. The problem then arose as to how *B. tumefaciens* could influence the chromosomes in meristematic tissues. This influence would appear impossible unless there were a large number of bacteria in the close vicinity of or within the actively dividing cells. However, it has been proven by many authors that the number of bacteria introduced in a single inoculation did not necessarily affect the size of the gall on the stem. In fact, even single bacteria when introduced into needle punctures of various sizes induced gall formation (11). Nevertheless a single bacterium introduced by Hildebrand into a single living cell failed to produce a gall or to survive. It appears that the bacteria alone do not produce the stimulation necessary for the growth of gall. It is only in combination with a factor such as mechanical injury that the bacteria can infect and cause overgrowth. On *Allium cepa* a needle-prick wound or freshly scraped surface of the stem were prerequisites for infection.

The reaction to infection in *Allium cepa* bulbs was a rather vigorous growth in a region distinct from the region of inoculation. In most crown

galls, true galls arise at the needle prick where *Bacterium tumefaciens* is introduced. It has been suggested by many authors, among them Levine (21), that crown gall is thus a protective reaction of the plant against the invading parasite. A tissue is produced which in the early stages appears uniformly embryonic but becomes characteristically differentiated as it grows older. In *A. cepa* the growth is away from the infected region and the plant does not form tissue to block the infection at the point of inoculation.

Recently there have been many studies in which factors involved in nonbacterial gall formation were investigated. Among these, Flint and Moreland (8), who studied gall formation in decapitated young bean plants, noticed that the healing wound overgrowths which resulted from decapitation developed independently of such gall-inducing practices as the application of indoleacetic acid or of *Bacterium tumefaciens*. On *Allium cepa* the sterile needle prick was not sufficient to produce a growth response.

The use of other agents gave varying results. A very harsh treatment consisting of the injection of a minute quantity of glacial acetic acid in the point where the needle-prick inoculation was ordinarily performed resulted, before the death of the plant, in a spreading of the still-living stem tissue in all directions and a breaking of connection between the injured region and the periphery of the stem (Fig. 8). There was possibly no time for any growth response in the leaves since the very toxic agent caused the early death of the plant. But even in this drastic treatment the effect was fairly distributed around the circumference of the stem. Lanolin paste containing 0.2 per cent indoleacetic acid caused a vertical elongation of the stem which had been as symmetrical as the bulb itself at the beginning of the experiment (Fig. 7). Certain irregular growth responses were obtained with agents other than *Bacterium tumefaciens*. Deep cuts severing great parts of the stem from the bulb produced irregular growth formations at the base of the bulb (Fig. 9). The application of diethylstilbestrol directly to the stem base produced a similar irregular growth response (14). Thus in one case a physical agent and in the other a chemical agent produced a certain deformity. The deformity caused by diethylstilbestrol resembled to a certain degree the response of the bulb to *B. tumefaciens* inasmuch as the stem widened in one of the horizontal diameters. There was, however, no similarity between the diseased condition of the *B. tumefaciens*-treated bulb and the healthy and vigorous appearance of the diethylstilbestrol-treated bulb. It cannot be said at present whether diethylstilbestrol acted in a colchicine-like manner in the production of these plant overgrowths (19).

Although it is difficult to analyze all the factors responsible for overgrowth in *Allium cepa*, it seems necessary to take into consideration the activity of terminal and lateral buds in the interior of the bulb. The experiments seemed to indicate that the deformity which arose on plants which had been deeply cut was due to excessive leaf growth which involved not

only the terminal but also the lateral buds. It is possible that the lateral bud was stimulated in bulbs infected by *Bacterium tumefaciens* and in those treated with diethylstilbestrol. In *Nicotiana* hybrids (37), the greatly increased activity of the lateral axillary buds when the plant reached a certain physiological age resulted in, and seemed to be the main factor in, tumor formation. In the case of overgrowths on *A. cepa* the stimulus of bacteria or of diethylstilbestrol might have upset some regulating mechanism controlling the activity of the lateral bud.

The overgrowth response of *Allium cepa* consists roughly in an increase in number of parenchyma cells. Histologically it resembles the numerous wound overgrowths and chemically produced overgrowths on decapitated plants which were reported during recent years by many authors. Among these are Whiting and Murray (38), who studied the histological response to such agents as nicotine and wounding. In all these cases the overgrowth tissues consisted of parenchymatous cells. In the present investigation it was possible to secure some evidence of divisions among the cortical parenchyma cells in *A. cepa* which had been treated with *Bacterium tumefaciens*. There has been, however, no confirmation that polyploidy is a predominant feature of the crown gall disease. Evidence brought forth in this investigation indicated that the nature of the cortical parenchyma cells which are in a polyploid condition but not destined to divide could be revealed as polyploid by a combination of factors, one of which was *B. tumefaciens*. It seems that some early reports on polyploidy in connection with crown gall might be interpreted in much the same manner.

SUMMARY

Bulbs of *Allium cepa*, when grown in tap water, are susceptible to *Bacterium tumefaciens* introduced by needle-prick inoculation.

An abnormal localized overgrowth of the bulb, which did not closely resemble any of the reported cases of crown gall, has been obtained in 30 cases in about 13 days after inoculation. The application of diseased tissue to healthy plants produced infection in 15 cases. Histological and cytological features of stages of the infection, involving mainly progressive necrosis, have been described. The roots and seedlings of *Allium cepa* were immune from the action of the bacteria.

No confirmation that polyploidy is a predominant feature or factor of crown gall disease was obtained. The evidence suggested that the cortical parenchyma cells of the stem may be in the polyploid condition in the uninfected bulb although not destined to divide. A combination of factors, one of which is *Bacterium tumefaciens*, may reveal polyploidy by stimulation of cell division.

Growth response of *Allium cepa* to indoleacetic acid, podophyllin, glacial acetic acid, diethylstilbestrol, and to partial excision of the stem has been investigated.

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SEED TREATMENT OF FIELD LEGUMES

A . J . V l i t o s [1] A N D D . A . P r e s t o n [2]

INTRODUCTION

The value of chemical seed treatments for numerous types of vegetable seed as a means of protection against damping-off has become well recognized. However, very few definite experimental data have been forthcoming to substantiate the value of such treatment in increasing stands and improving germination of the field legumes, and little is known of the effects of chemical treatment on nodulation.

A common practice of farmers has been to plant legume seed treated only with bacterial inoculum ("Nitragin," etc.), no further treatment or protection being thought necessary; but under this practice seeding rates for such crops as alfalfa and clovers have been excessively heavy because of poor stands and the large number of damped-off seedlings. A further belief, fostered by commercial publicity, has been that bacterial inoculum and seed treatment chemicals are incompatible, with the result that growers have avoided chemical treatments. These assumptions did not appear to have a factual basis, and experiments were undertaken at the Oklahoma Agricultural Experiment Station to clarify the problem. The experiments were begun in September, 1946, and continued through July, 1948, under joint sponsorship of the Naugatuck Chemical Division of the United States Rubber Company and the Oklahoma Agricultural Experiment Station.

REVIEW OF LITERATURE

Favorable results have been obtained from seed treatment of alfalfa in Iowa (4), alfalfa and clover in New York (7), alfalfa, lespedeza, and clovers in Pennsylvania (5, 9), and peanuts in several Southern States. Conflicting results have been obtained in tests with clovers in Wisconsin (2) and with soybeans at numerous places (1). General conclusions or recommendations probably should not be drawn from these limited data, particularly since tests have not been made on a number of types of field legumes.

The literature concerning the effects of chemical treatment on nodulation also has been relatively scanty and conflicting. Appleman (3) found

that satisfactory nodulation was produced upon pea and soybean plants grown from inoculated seed treated with Semesan. Cuprocide, in his tests, prevented nodulation on soybeans and canning peas. Ceresan decreased nodulation on canning peas but not on soybeans. All nodulations appeared to be on lateral roots where chemicals were used, but taproot nodulation was obtained on plants from nontreated, inoculated seed. Kadow, Allison, and Anderson (8) found a decrease of nodulation from an average of 75 nodules per plant (nontreated) to 3 and 4 nodules per plant treated with organic mercury, cuprous oxide, and zinc oxide. Duggar (6) has demonstrated nodules forming on plants from seed treated with Semesan, copper sulfate, saturated boric acid, bichloride of mercury, and concentrated sulfuric acid. Some of the treatments decreased nodulation, while others increased it. Buchholtz (3) reported that small-seeded legumes grown from seed treated with organic mercury dust bore root nodules when planted in the field. Some of the earliest work done by Miller and Stapp, reported by Appleman (3), in Germany demonstrated that if nodule bacteria are in the soil at the time of planting, seed treatment chemicals do not hinder the development of root nodules.

Conflicting reports are found in Litynski's (10) work in Poland, where he observed that some treatments reduced nodulation, and at other times the same treatments had little or no apparent effect on nodulation.

MATERIALS AND METHODS

Certain methods and procedures that were employed throughout the investigations are described here. Variations from the procedures occurred at times, and those deviations will be discussed later. Replicated field and greenhouse experiments were made with Austrian winter pea, mungbean, Chinese red cowpea, yellow hop clover, hairy vetch, and alfalfa. Seed of these were treated with Spergon (tetrachloro-p-benzoquinone), Phygon (2,3-dichloro-1,4-napthoquinone), Arasan (tetramethylthiuram disulfide), Dow 9B (zinc trichlorophenate), and Ceresan M (ethylmercuric-p-toluene sulfanilide). Nontreated controls, seed inoculated with "Nitragin" bacterial inoculum, and noninoculated seed were included. In all but one of the trials in 1946–1947, the seed were uniformly treated with all the chemical that would adhere to the seed coats; however, in the experiments of 1947–1948 seed was treated at 0.25, 0.50, and 1.00 per cent dosage rates by weight of seed. In the field tests (1946–1947), 5 replications of 200 seed each were used in a randomized block design, for each crop. In the greenhouse the treated seed were inoculated with "Nitragin" and planted in flats or pots. Five replications of 100, 50, or 25 seed, depending upon the crop, were planted in a randomized block design for each crop.

In all cases the application of the desired amount of chemical upon the seed was accomplished by shaking seed and chemical together in glass

tumblers. "Nitragin" was used in a dry form and shaken over the treated seed before planting in sterile soil. Soil was sterilized by autoclaving for 3 hr. at 15 lb. pressure.

The average greenhouse temperature for spring and fall was 70° F.; for summer, 85° to 90° F.

EXPERIMENTAL RESULTS

The mean numbers of seedlings produced from treated and nontreated seed for each of the crops tested in the field and greenhouse are given in the summary tables. The mean numbers of plants producing nodules are also included. The analysis of variance for each of the crops was calculated, and appears in summary form. The experimental data for each plot are on file in the Department of Botany and Plant Pathology of the Oklahoma Agricultural Experiment Station.

TABLE 1.—*Effect of seed treatment on the field stand of legumes, Stillwater, Oklahoma, 1946–1947*

Seed treatment	Mean number of plants emerged[a]				
	Austrian winter pea	Mungbean	Chinese red cowpea	Yellow hop clover	Hairy vetch
Spergon	124	89	116	81**	106
Phygon	141	80	104	67**	133
Arasan	153	102	131	40**	163
Dow 9B	151	112	115	51**	146
Ceresan M	116	76	114	25	132
Nontreated	140	97	107	21	149
LSD at 0.05	24	20		12	
LSD at 0.01		27		16	

[a] Averages for 5 replicates of 200 seeds each.
** Highly significant at 0.01 level.

The Austrian winter pea data are presented in tables 1, 2, and 3. In general, the seed treated with Spergon, Phygon, Arasan, and Dow 9B produced more seedlings than nontreated seed, although not significantly so (Table 1). Plants produced from seed treated with these chemicals also produced more nodules, significantly so with the Arasan treatment. Seed treated with Ceresan M produced fewer seedlings than nontreated seed.

The mean numbers of Austrian winter pea plants produced from seed treated with the different chemicals at varied dosage rates (Table 3) indicate the dosage levels at which the respective chemicals were most effective on this seed. Spergon, Phygon, and Dow 9B were most effective when applied at 1.00 per cent by weight of seed, and Arasan was most effective at 0.50 per cent. The results indicate that the chemicals should not be used in excess of the rates of application indicated.

The mungbean data for 1946–1947 (Table 1) indicate that Spergon, Phygon, Arasan, and Dow 9B were effective chemicals in comparison to the nontreated controls. Further tests conducted with mungbean in

greenhouse trials in 1947–1948 (Table 3) showed all treatments highly significant over nontreatment, with the exception of Spergon at the 0.25 and 0.50 per cent dosage rate. Phygon at 1 per cent gave the highest mean average (33 plants), and in general the plants resulting from seed treated with this chemical were strikingly taller and healthier looking than seedlings from seed treated with the other chemicals. There was some hardening of seed coats with Phygon-treated seed at the highest dosage.

Spergon, Phygon, Arasan, and Dow 9B improved the emergence of cowpea var. Chinese Red in greenhouse tests conducted in 1946 (Table 2) in significantly better germination than nontreatment. Significantly more plants with nodules resulted from these chemical treatments than from nontreatment. The same trend was evident in the field germination trials. In the greenhouse trials in 1948 (Table 3), Phygon, Spergon, Arasan, and Dow 9B at all dosage rates were highly significant over nontreatment.

TABLE 2.—*Effect of seed treatment on stand and nodulation of legumes in the greenhouse, Stillwater, Oklahoma, 1946–1947*

Seed treatment	Number of plants emerging and nodulated*				
	Austrian winter pea	Chinese red cowpea	Mungbean	Hairy vetch	Yellow hop clover
Spergon	80–76	87**–80**	80**–78**	74* –71**	85–81**
Phygon	82–77	90**–84**	77**–74**	79**–76**	79–75**
Arasan	87–86*	83**–80**	69**–64**	76* –70**	72–69*
Dow 9B	86–82	78**–75**	71**–66**	82**–79**	68–62
Ceresan M	15–12	51 –49	7 – 4	53 –49	58–53
Nontreated	77–73	57 –52	30 –22	64 –57	59–54
LSD at 0.05	14–11	10 –11	14 –13	9 – 9	8–11
LSD at 0.01	19–15	13 –15	20 –18	12 –12	11–15

* Averages for 5 replicates of 100 seeds of Austrian winter pea, Chinese red cowpea, mungbean, and yellow hop clover. The left figure of each pair refers to the number of plants emerged, the right one to the number with nodules.
* Significant at 0.05 level.
** Highly significant at 0.01 level.

Spergon appeared to be very effective on yellow hop clover seed (Table 1). In the field trials, Spergon, Phygon, Arasan, and Dow 9B were each slightly significantly better than either of the other three chemicals. In the greenhouse trial, nontreatment was significantly poorer than treatment with Spergon, Phygon, Arasan, and Dow 9B. Treatment with Spergon was significantly better than with Arasan and Dow 9B. More of the plants produced from the treated seed formed nodules than from the nontreated seed. Nodule formation was lateral, in most cases, on plants which were chemically treated. Spergon was outstanding as to nodule formation on plants resulting from seed treated with this chemical.

The results with hairy vetch (Tables 2, 3) in the greenhouse were promising, but the field data were too variable for confirmation. In one greenhouse test (Table 2) in 1947, seed treated with Spergon, Phygon, Arasan,

and Dow 9B produced significantly more plants than nontreated seed and significantly more of these plants formed nodules. In 1948 a greenhouse experiment (Table 3) revealed that plants produced from Arasan-treated seed were significantly higher in germination than nontreated seed. There was also very evident damage by Dow 9B at all dosages. Spergon at 0.25 dosage rate, and Phygon at the 0.25 dosage rate were significant over non-treatment. The experimental results with alfalfa are clear-cut, as is apparent from tables 3, 4, and 5. Tables 3 and 4 give germination figures.

TABLE 3.—*Effect of seed treatment and dosage on germination of legumes in greenhouse experiments, Stillwater, Oklahoma, 1947–1948*

		Mean number of plants[a]				
Chemical	Dosage	Austrian winter pea	Chinese red cowpea	Mungbean	Hairy vetch	Alfalfa
Spergon	0 00	83	17	6	25	10
	0.25	89*	21**	8	32	27**
	0.50	91**	22**	13	28	33**
	1.00	94**	22**	15*	17	27**
	Excess	81				
Phygon	0.00	83	17	6	25	10
	0 25	84	21**	23**	32	58**
	0.50	85	20**	32**	31	63**
	1 00	95**	23**	33**	26	61**
	Excess	87				
Arasan	0.00	83	17	6	25	10
	0.25	83	20**	19**	38	40**
	0 50	94**	20**	20**	49**	40**
	1 00	87*	22**	25**	42**	30**
	Excess	82				
Dow 9B	0 00	83	17	6	25	10
	0.25	78	21**	19**	5	15
	0.50	83	22**	32**	0	9
	1.00	92**	20**	18**	0	13
	Excess	74				
LSD between chemicals for whole test						
at 0.05 =		4.00	2.00	8.25	8.0	6.9
at 0.01 =		6.00	2.66	11.04	10.4	9.2

[a] Averages for 5 replicates of 100 seeds each of Austrian winter pea and alfalfa, 50 seeds each of mungbean and hairy vetch, and 25 seeds of Chinese red cowpea.
** Highly significant at 0.01 level.
* Significant at 0.05 level.

Table 5 reports independent experiments investigating nodulation-chemical relationships.

In the first of five replications on November 8, 1947 (Table 3), it was found that Phygon at all dosages, Arasan at all dosages, Dow 9B at 0.25 per cent, and Spergon at all dosages, all were significantly superior to non-treatment. Phygon was the outstanding treatment. There was some damage to seed coats by Dow 9B at the highest dosage rate.

Immediately following the highly significant results of the first test with alfalfa, another five replications were planted on December 15, 1947. Phygon-treated seed was significantly better again at all dosage rates and Arasan-treated seed was significantly better at the highest and lowest dosages. No other chemicals proved significantly superior to nontreatment. One hundred seeds per treatment were planted. On January 5, 1947, another five replications were planted, and once more Phygon showed convincing significance at two dosage rates (0.50 and 1.00 per cent by weight of seed). Arasan was superior with high significance at the 1.00 per cent dosage rate, Dow 9B at the 1.00 dosage being significantly better than nontreatment at the 0.05 LSD level. In this test all plants resulting from Phygon-treated seed appeared to have made a more vigorous growth than those from seed with other treatments. In additional tests with alfalfa an attempt was made to correlate some of the results with nodulation-chemical relationships. Table 4 gives first-recorded germination figures. Phygon-

TABLE 4.—*Effect of seed treatments on alfalfa germination*

Treatment	Dosage rate	Mean number of plants[a]
Check—not inoculated		25.6
Check—inoculated		26.8
Phygon—not inoculated	1.00	36.6[**]
Phygon—inoculated	1.00	38.4[**]
Arasan—not inoculated	1.00	30.8
Arasan—inoculated	1.00	30.6

LSD at 0.05 level = 5.21
LSD at 0.01 level = 7.11

[a] Averages for 5 replicates of 50 seed per treatment.
[**] Highly significant at 0.01 level.

treated alfalfa seed yielded a highly significant number of emerged plants as compared with nontreated seed. Arasan-treated seed (noninoculated) was significant in its yield of plants as compared with nontreatment. There were fifty seeds per treatment.

Table 5 consists of data showing the number of plants having nodules developed. In all cases the nodules were formed on lateral roots. In the calculation of percentage figures, the germination figures in table 4 were used, and employing transformation tables (Hayes and Immer), it was possible to carry out an analysis of variance for nodulation. The results are relatively conclusive. With a mean of 60.6 for Phygon-inoculated seed and a mean of 55.4 for Arasan-inoculated as compared with 37.4 for check-inoculated, it is evident that chemical treatment enhanced nodule formation in this test. Both the Phygon and Arasan figures for nodulation are highly significant over nontreated seed.

Table 5 also lists the results obtained with Chinese red cowpea seed treated with Phygon, Spergon, and Arasan (all at the 1.00 per cent dosage

rate), and the effects of these chemicals on nodulation. It is evident that nodulation was not inhibited by chemical treatments, even if treated seed was inoculated with "Nitragin" bacterial inoculum. Nodulation in this case was on lateral roots of plants resulting from chemically treated seed.

DISCUSSION OF RESULTS

Of the legumes tested, alfalfa, yellow hop clover, mungbean, and hairy vetch responded most consistently to seed treatment. On yellow hop clover, Spergon treatment was significantly superior to nontreatment and also to either Arasan or Dow 9B treatment in both field and greenhouse trials. Spergon, Phygon, Arasan, and Dow 9B were each significantly superior to nontreatment.

TABLE 5.—*Compatibility of seed treatment and inoculation with nodule bacteria, and the effect on nodulation, in alfalfa and cowpea*

Seed treatment	Inoculation with nodule bacteria	Mean number* of plants with nodules	
		Alfalfa	Cowpea
Spergon, 1 per cent	Inoculated		63.6**
Spergon, 1 per cent	Not inoculated		0 08
Phygon, 1 per cent	Inoculated	60 6**	71.2**
Phygon, 1 per cent	Not inoculated	0 0	0.0
Arasan, 1 per cent	Inoculated	55.4**	71.2**
Arasan, 1 per cent	Not inoculated	0.08	0 0
Control	Inoculated	37.4**	53.2**
Control	Not inoculated	0.06	4.5
	LSD at 0.05 =	7.91	26.19
	LSD at 0.01 =	10 70	34.04

* Averages for 5 replicates of 100 seeds of alfalfa and 100 seeds of Chinese red cowpea.
** Highly significant at 0.01 level.

The results obtained with the seed treatment of alfalfa were outstandingly consistent. Treatment with Phygon, which, prior to this work, had not been extensively tested on legumes, proved superior to nontreatment with high significance, and Arasan at the highest dosage also increased germination significantly. Hardening of seed coats, a characteristic that is associated with legume seed treated with heavy dosages of Phygon, did not affect the germination of alfalfa seed treated with 1.00 per cent dosages of Phygon. With three highly significant confirmatory tests conducted under nearly identical conditions, there is evidence of value in treating alfalfa seed with Phygon.

Mungbean, which is being grown on increasing acreages in Oklahoma, responded significantly to seed treatment with Phygon, Dow 9B, and Arasan, all of these treatments yielding highly significant results over nontreatment. Phygon, Arasan, and Dow 9B were also significantly superior to Spergon on mungbean seed. Phygon-treated seed yielded plants that

were strikingly taller and healthier in appearance than seed treated with Arasan, Dow 9B, or Spergon.   It is also interesting to note the low germination figure of nontreated seed.

Hairy vetch seed treated with Arasan at all dosages, Phygon at 0.25 dosage, and Spergon at 0.25 dosage were significantly superior to nontreatment and to Dow 9B-treated seed.   There was damage to seed treated with Dow 9B, appearing as disintegration and charring of the seed coats.

There was no indication in any of the tests with the legumes that chemical treatment of these seed inhibited nodule formation; there was, however, considerable evidence that nodulation is enhanced by Phygon, Arasan, Spergon, and Dow 9B with the majority of these seed.   In the tests with alfalfa, Phygon- and Arasan-treated seed increased nodulation considerably, although it is interesting to find that the nodules were formed extensively on lateral roots, rather than on the tap root.   This type of nodule formation was evident on the other legume seed treated with chemicals in all of the tests performed.   On seed not treated with chemicals, nodules were formed on tap roots as well as on lateral roots.

SUMMARY

Alfalfa seed treated with Phygon at 1 00, 0.50, and 0.25 per cent dosages by weight of seed gave highly significant germination as compared with nontreated seed.   Arasan at 1.00 per cent, Dow 9B at 1.00 per cent, and Spergon at 0 50 per cent dosage rates were all superior to nontreatment, but less effective than Phygon.

Mungbean seed treated with Phygon, Arasan, Dow 9B, and Spergon responded significantly to treatment, with Phygon at all dosages being most effective and yielding plants that were taller than seedlings from seed treated with other chemicals.

Chinese red cowpea seed treated with Arasan, Phygon, Spergon, or Dow 9B gave higher germination figures, significantly so, over nontreated seed.

Yellow hop clover seed responded most consistently to Spergon treatment as compared with Arasan, Phygon, Dow 9B, and Ceresan M treatments.

Hairy vetch seed showed highest germination where treated with Arasan, although Phygon-treated seed also was significantly superior, in germination, to nontreated seed.

Ceresan M proved to be injurious to all of the legume seed that were treated with this volatile chemical, and as a result was not included in experiments carried on in 1948.

Nodulation was not inhibited by chemical treatment of seed of alfalfa, mungbean, yellow hop clover, Chinese red cowpea, hairy vetch, and Austrian winter pea, when chemical treatment was followed by seed inoculation with ''Nitragin'' bacterial inoculum.

Nodulation of alfalfa and Chinese red cowpea was enhanced if seed of these legumes were treated with Phygon or Arasan and then inoculated

with "Nitragin." Chemically treated seed yielded more plants with nodules than nontreated seed.

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DYING OF LIVE OAKS IN TEXAS[1]

A . A . D U N L A P A N D A . L . H A R R I S O N

The death of live oaks (*Quercus virginiana*) in Texas has been observed or reported in some twenty counties in the central part of the State, and has been called to the attention of plant pathologists over several years. The dying of 200 live-oak trees in the vicinity of Austin during 1933 and 1934 was described as the "live-oak disease" by Taubenhaus;[2] trees continued to die in that area in 1935. The nature of the causal agent has not been determined. Because the live oak is a valuable shade tree, the disease is of much economic importance in areas of its occurrence.

The environment in which the trees are growing appears to have little, if any, effect on the disease. Complaints of the dying of live oaks usually concern valuable trees near residences or trees that provide shade for livestock in farmyards (Fig. 1, A), although the disease is not restricted to these localities. It has been observed in forest land and even among trees near creeks where there is an abundance of moisture throughout the year. In surveys made in 1941 and 1942, covering several counties in south-central Texas, affected trees were found on both acid and highly alkaline soils, on sandy and heavy clay soils, on dry and wet soils, on hills and in valleys. Some of the trees were covered with Spanish moss and some were not. Young trees as well as older ones are affected. The disease occurs in somewhat restricted local areas and it seems to spread slowly to neighboring trees either in the same clump or in the same general locality.

Symptoms of the live-oak disease consist of yellowing or mottling of the leaves followed by death of certain branches or of the whole tree. Oftentimes the entire tree apparently becomes affected and it may die within a few weeks. If a tree is affected in only one branch at a time, one or two seasons may elapse before the entire tree is killed. In some instances a tree may appear healthy at the time of leaf shedding in early spring, but fail to put out new leaves at the usual time a few weeks later. Streaks of discolored wood have sometimes been seen in the roots of dying trees. Sprouts may appear from the roots of trees whose tops have died recently, but usually there is no persistent new growth by means of root or trunk sprouts.

Laboratory cultures of various parts of dying trees, including the discolored root tissues, have yielded no organism that has been proved or considered to be pathogenic; nor have a few grafting and budding experiments resulted in definite transmission of the symptoms. Studies of the dis-

[2] Taubenhaus, J. J. Live-oak disease at Austin. Tex. Agr. Exp. Sta. 47th Ann. Rept., pp. 97–98. 1934. Also 48th Ann. Rept., pp. 99–100. 1935.

ease made thus far suggest that the trouble may be due to a virus, but this is purely conjecture.
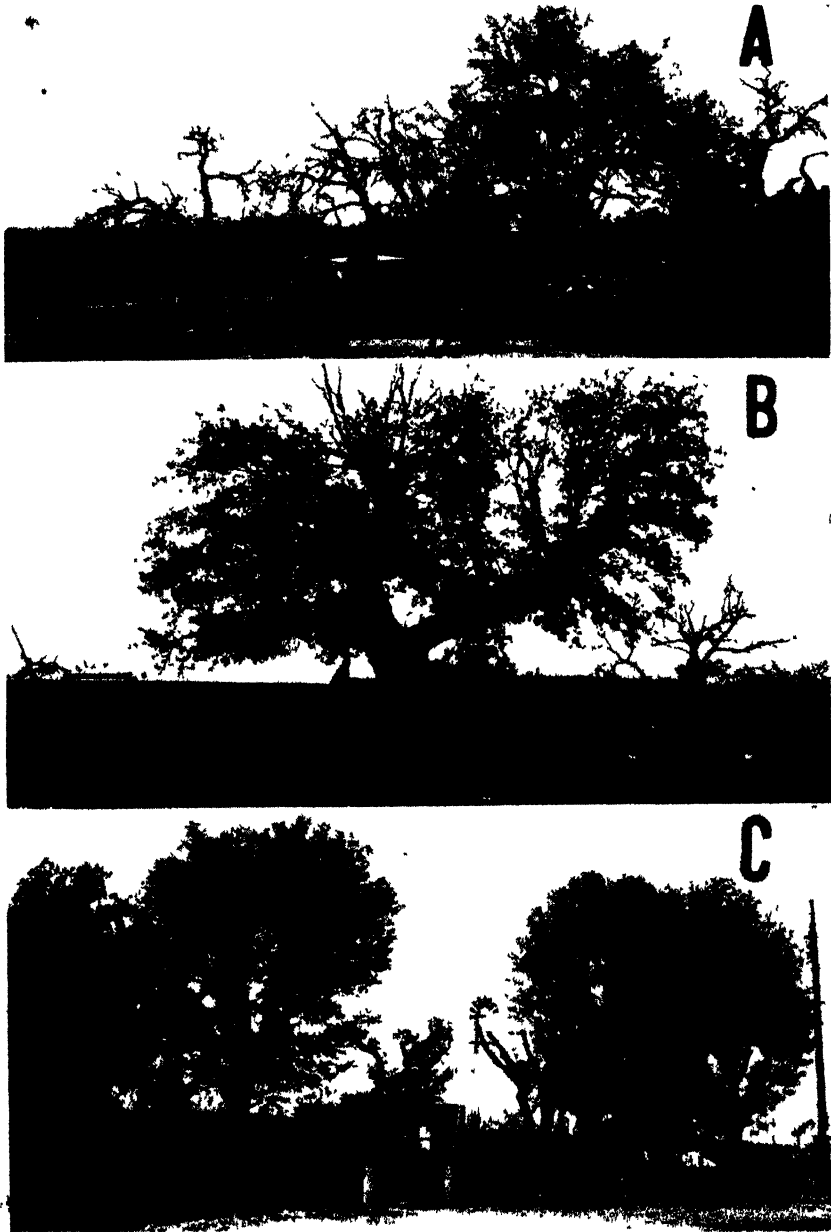
FIG. 1. Effects of pruning on death of live oaks in one farmyard. A. Only one tree remains alive in this group of seven live oaks which were not pruned. B. Large live oak that was topped when threatened with death in 1940. Dead nonpruned tree in background. C. New healthy tops formed on live oaks that were pruned when threatened with death in 1940. One trunk (center) is still alive although sprout growth was poor. One forked trunk (right center) failed to form sprouts after the pruning. Photographed April, 1948.

During the late fall of 1940, attention was called to a serious outbreak of the disease in Bosque County, near Clifton, Texas. At that time some experimental pruning was undertaken in an attempt to check the dying of live oaks around two ranch homes. The larger limbs were removed, in most cases, leaving only the main trunk of the tree standing (Fig. 1, B). The topped trees were observed from time to time, and, while some live oaks continued to succumb in the same general localities, only a very few trees died of the several that were severely pruned. Records taken in April, 1948, of the trees that were topped in November, 1940, showed that strong sprout growth from the treated trees had resulted in partial restoration of the top. Photographs of some of the trees, taken about 7½ years after severe pruning, are shown in figure 1, B and C. Among the 20 or more trees that were topped in this fashion only three were dead at the time of this last inspection. It is presumed that the disease was too far advanced in these three trees at the time of topping to permit recovery following the treatment (Fig. 1, C).

A resident of Yoakum, Texas, had lost four large live-oak trees near his residence previous to 1948. In the spring of that year, two more of the remaining live oaks also failed to produce new leaves following the usual defoliation in the late winter. One of these trees was pruned back to the main trunk late in April and the other was not pruned. During the summer new shoots appeared on the pruned trunk and on the stubs of the large branches. The nonpruned tree died completely without showing any growth of sprouts from the trunk.

The significance of the apparent recovery of the topped live-oak trees is not known but it is suggested that this treatment might prove effective against similar disorders in other woody plants.

SUMMARY

Live oaks have been observed to die more or less suddenly under a variety of environmental and seasonal conditions in central Texas. No causal agent has been associated with the so-called disease. Severe pruning of the main branches, at first appearance of symptoms, has apparently resulted in recovery and continued health of the trees in many instances.

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ROUGH BARK OF TUNG, A VIRUS DISEASE

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In 1945 a tung tree, *Aleurites fordii*, having peculiar rough, black bark on the trunk and limbs and long, slender, twisted, willowy branches, was found in a tung orchard in St. Tammany Parish, Louisiana. An early symptom of this "rough bark" disease is a light bronze discoloration of the bark followed by the development of small widely spaced blisters on the surface. Later the bark turns dark in color and cracks into small irregularly shaped segments (Fig. 1, A). The diseased bark is in striking
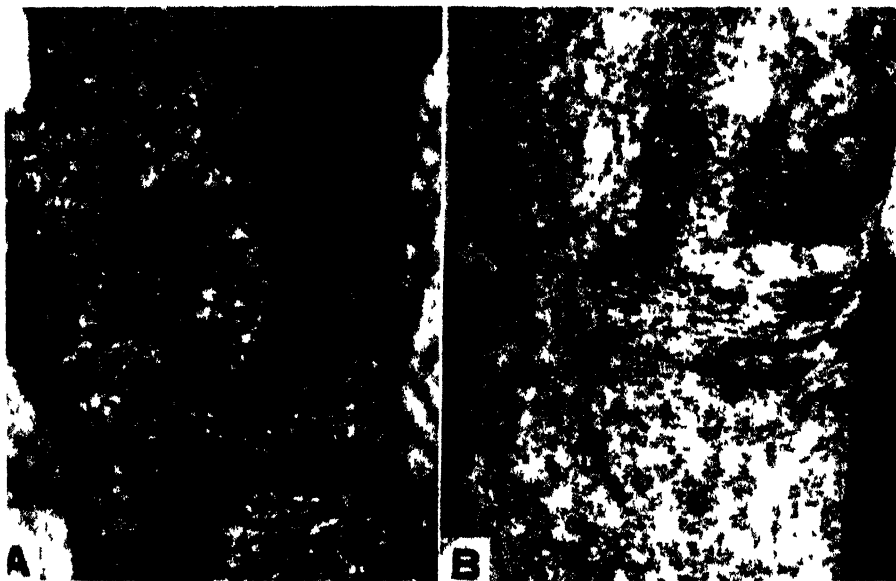
FIG. 1. A, tung tree affected with "rough bark" disorder. B, normal tung tree.

contrast to the smooth, light gray bark of a normal tung tree (Fig. 1, B). A second tung tree affected with rough bark disease was found in Pearl River County, Mississippi, in 1946.

Since the writer has found only two tung trees in the southern United States that are affected, the disease is not of present economic importance. Its effect upon the trees is difficult to judge, because they are seedlings and subject to much inherent variation. However, one of the trees is small and stunted, and both have long twisted internodes. Foliage is a normal dark green without variegation or mottling, but both trees appear to be subnormal in growth and production.

The tissues and buds of affected plants have been examined for a possible insect infestation or causal organism, fungus or bacterium, but none has been found. It was therefore considered that the abnormality might be of genetic origin or caused by a virus.

To determine whether the disorder is caused by a virus, two budding experiments were started in 1946. In April, buds taken from the rough bark tree in St. Tammany Parish, Louisiana, were inserted by the T-bud method on 100 normal tung seedling rootstocks. On the same date, buds from normal trees were set in an equal number of normal tung rootstocks. The rootstocks were cut back to about 6 in. above the implanted buds in July, 1946. In September, 40 more rootstocks were budded with buds from the rough bark tree, and 20 with buds from normal tung trees. In May, 1947, the fall-budded rootstocks were cut back to about 6 in. above the implanted buds. The object was to force the implanted buds, but two or three suckers were permitted to grow from each rootstock to test the transmission of the disorder from the bud to the healthy rootstock. Observations on the 260 budded tung trees during 1947 and 1948 are summarized in table 1.

TABLE 1.—*Transmission of rough bark disease in tung trees budded in 1946*

Type of bud	Rootstock budded	Bud shoots as of November, 1948		Sucker shoots as of November, 1948[a]	
		Alive	With definite rough bark symptoms	With definite rough bark symptoms	With incipient rough bark symptoms
	Number	*Number*	*Number*	*Number*	*Number*
Rough bark	140	43	25	25[b]	18
Normal	120	40	0	0	0

[a] There were on the average 2 or 3 suckers per rootstock.
[b] Of the affected suckers, 14 arose above and 11 below the implanted bud.

The rough bark symptoms were slow to appear on the budded trees; the first definite symptoms were observed on the bud shoots in the second season, 1947. Four diseased suckers were also observed, two growing from above and two from below the implanted rough bark bud. In November of the third season, 1948, twenty-five of the bud shoots had definite rough bark symptoms. Fourteen sucker shoots that grew from above the implanted buds and eleven that grew from below developed rough bark symptoms. Eighteen more sucker shoots were observed developing the bronze bark appearance, characteristic of the early stages of the disease. No disease symptoms were observed on the trees budded with normal buds.

The transmission of the rough bark disease from the bud through the healthy rootstock to suckers developing above and below the implanted bud indicates that this disorder is a virus disease. To date no studies on possible insect vectors have been made.

SUMMARY

A disease that causes rough bark and willowy twisted branches of tung, *Aleurites fordii* Hemsl., is described. The disease, which has been named "rough bark," has been found in two widely separated orchards, one in Louisiana and one in Mississippi. Implanting buds from diseased shoots into healthy stocks has induced the disease in suckers developing from the rootstocks both above and below the implanted rough bark bud. In the absence of other causal organisms, the transmission of rough bark disease from the affected bud through the healthy rootstock to suckers indicates that this disorder is a virus disease.

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THE FUNGICIDAL ACTIVITY OF SUBSTITUTED PYRAZOLES AND RELATED COMPOUNDS

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Several types of nitrogenous compounds are fungitoxic. Among the more active are the quaternary ammonium compounds, 8-hydroxyquinoline derivatives, glyoxalidines, thiazoles containing salt-forming groups, and N-nitrosophthalimidine. All of them, except some of the quaternary ammonium compounds, contain the nitrogen in a heterocyclic ring.

In the course of a fundamental study on fungicides made in 1945, another group of heterocyclic nitrogen compounds was found to be active. These compounds, commonly known as pyrazoles, have the following nuclear structure:
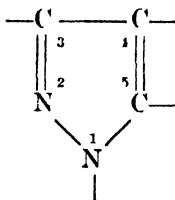
Substitution of a nitroso group in the 4-position enhanced the activity of the pyrazoles, particularly of those compounds with an aryl group in the 1-position (27). Over 100 compounds were synthesized and tested in determining these relations. Data are presented on the performance of selected members in laboratory and greenhouse tests made to determine the effects of modification of the pyrazole nucleus, substitution in the 4-position, substitution in the 1-position, and substitution on a phenyl group in the 1-position on fungicidal and fungistatic activity. Data are also presented on the field performance of selected pyrazoles as seed, foliage, and fruit protectants.

PREPARATION AND PROPERTIES OF COMPOUNDS TESTED

Only about one-third of the pyrazoles and related compounds used in defining the active groups are reported in this paper. These are representative and clearly demonstrate the relations found. Insofar as could be determined by the usual standards, these were, with only three exceptions, pure compounds. The names and outstanding characteristics of these compounds are listed in table 1.

Pyrazoles can be prepared by reacting hydrazine or a monosubstituted

hydrazine with a beta dicarbonyl compound. For example, the reaction
of *p*-chlorophenylhydrazine and acetylacetone proceeds as follows:

1-*p*-Chlorophenyl-
3,5-dimethylpyrazole
(J54)

By choice of different monosubstituted hydrazines, the pyrazole molecule
can be varied in the 1-position. By use of different beta dicarbonyl com-
pounds, the substituents in the 3- and 5-positions can be modified. A nitroso
group can be introduced into the 4-position by nitrosating the dicarbonyl
compound before its reaction with the hydrazine:

$$CH_3COCH_2COCH_3 + HNO_2 \rightarrow CH_3COCCOCH_3 + H_2O$$

$$\underset{NOH}{|}$$

Isonitroso-
acetylacetone

1-Phenyl-3,5-dimethyl-
4-nitrosopyrazole
(J55)

Although three 4-nitrosopyrazoles were first prepared by this method
in 1902 by Wolff *et al.* (31), one of these compounds probably was prepared
by Knorr and Laubmann (14) as early as 1888 by direct nitrosation of
1,3,5-triphenylpyrazole.

The brief procedure of Wolff *et al.* (31) for the preparation of J55 was
employed in preparing most of the 3,5-dimethyl-4-nitrosopyrazoles tested.
An aqueous solution of isonitrosoacetylacetone was added to a stirred solu-
tion of an equimolar amount of the hydrazine in an equivalent quantity
or more of hydrochloric acid diluted sufficiently to dissolve the hydro-
chloride formed. The reactions were allowed to proceed, generally under
cooling, until the separation of the product was complete (2 to 24 hr.).

TABLE 1.—*Properties of compounds investigated*

Code	Name	Appearance	Crystn. solvent[a]	M.p.[b] (or b.p.)	Calcd.	Found	Previous synthesis
				°C. (uncor.)	Pct.	Pct.	
30J	3,5-Dimethyl-4-nitrosopyrazole	dark blue ndls.	benzene	128			(31)
4K3	1,3,5-Trimethyl-4-nitrosopyrazole	blue ndls.	ligroin	80–81	N, 30.2	29.4	(25)
39J	3-Methyl-4-nitroso-5-phenylpyrazole	dark green crystals	dil. alc.	149–150 (dec.)[c]			(31)
J55	1-Phenyl-3,5-dimethyl-4-nitroso-pyrazole	blue turquoise crystals	alcohol	95.5–96.5			
0M6	1-p-Iodophenyl-3,5-dimethyl-4-nitrosopyrazole	green ndls.	alcohol	112	N, 12.8	12.6	
7K1	1-p-Bromophenyl-3,5-dimethyl-4-nitrosopyrazole	green ndls.	alcohol	123			(25)
J49	1-p-Chlorophenyl-3,5-dimethyl-4-nitrosopyrazole	green ndls.	alcohol	118	N, 17.8	17.7	
4L9	1-p-Fluorophenyl-3,5-dimethyl-4-nitrosopyrazole	fine blue-green ndls.	alcohol	116–117	N, 19.2	18.9	
35L	1-m-Chlorophenyl-3,5-dimethyl-4-nitrosopyrazole	light green crystals	alcohol	84	Cl, 15.1	15.3	
27L	1-o-Chlorophenyl-3,5-dimethyl-4-nitrosopyrazole	blue crystals	alcohol	117	Cl, 15.1	15.2	
3K4	1-(2,4-Dichlorophenyl)-3,5-dimethyl-4-nitrosopyrazole	green crystals	alcohol	89–90	N, 15.6	15.6	
3K3	1-(2,5-Dichlorophenyl)-3,5-dimethyl-4-nitrosopyrazole	green crystals	alcohol	99–100	N, 15.6	15.4	
4K5	1-Chlorophenyl-3 (or 5)-methyl-4-nitroso-5 (or 3)-n-hexylpyrazole	dark green oil			purity uncertain		
2K5	1-p-Tolyl-3,5-dimethyl-4-nitrosopyrazole	blue-green needles	alcohol	112			(25)
7K2	1-p-tert-Amylphenyl-3,5-dimethyl-4-nitrosopyrazole	green crystals	alcohol	92–93	N, 15.5	15.4	
9K5	1-p-Carboxyphenyl-3,5-dimethyl-4-nitrosopyrazole	light green crystals	alcohol	decompn. begins ca. 170[d]	N, 17.1	17.0	
8K2	1-p-Nitrophenyl-3,5-dimethyl-4-nitrosopyrazole	green ndls.	alcohol	131–132 (dec.)[e]	N, 22.8	22.2	
39L	1-p-Thiocyanophenyl-3,5-dimethyl-4-nitrosopyrazole	green ndls.	alcohol	105	N, 21.7	22.2	
36L	1-p-Sulfamylphenyl-3,5-dimethyl-4-nitrosopyrazole	green crystals	alcohol	decompn. begins ca. 189[f]	S, 11.4	11.7	
0M9	1-p-Xenyl-3,5-dimethyl-4-nitrosopyrazole	green crystals	alcohol	163 (dec.)[g]	N, 15.2	15.3	
19K	1-α-Naphthyl-3,5-dimethyl-4-nitrosopyrazole	pale blue crystals	alcohol	88–89	N, 16.7	16.6	
L63	1-Phenyl-3-methyl-4-nitroso-5-N-methylanilinopyrazole (not pure)	light green crystals					(19)

TABLE 1.—(Cont.)

Code	Name	Appearance	Crystn. solvent[a]	M.p.[b] (or b.p.) °C. (uncor.)	Analysis Calced. Pct.	Analysis Found Pct.	Previous synthesis
P90	Isonitrosoacetylacetone	white flat crystals	benzene-ligroin	76			(31)
16K	α-Naphthylhydrazine (not pure)	leaflets	water	113–115			(8)
5J0	p-Chlorophenylhydrazine hydrochloride	white crystals	abs. alc.	decomp. 225–226			(7)
E24	Phenylhydrazine[h]						(12)
49K	4-Nitrosoantipyrine	dark green crystals	alcohol	decomp. suddenly 200			(13)
35J	1,3-Diphenyl-4,5-pyrazoledione-4-oxime	orange ndls.	dil. ale.	209 (dec.)[1]			(2)
J30	1-p-Chlorophenyl-3-methyl-4,5-pyrazoledione-4-oxime	yellow-orange hairlike ndls.	dil. alc.	180 (dec.)			
J54	1-p-Chlorophenyl-3,5-dimethylpyrazole	liquid		(b6 146–147)	Cl, 17.2	17.2	
L67	1-Phenyl-3,5-dimethyl-4-nitropyrazole	white crystals	dil. ale.	103			(31)
14K	1-Phenyl-3,5-dimethyl-4-amino-pyrazole monohydrate	white ndls.	water	66–67			(22)
53K	1-Phenyl-3,5-dimethyl-4-phenylazopyrazole[h]	yellow ndls.	alcohol	62–63			(1)
1E1	3,5-Dimethylpyrazole	white ndls.	ligroin	65–66			(30)
9K8	3-Methyl-5-ethoxypyrazole	colorless liquid	ligroin	(b2 108–109)			(20)
8K3	1-Phenyl-3-methyl-5-chloropyrazole	white crystals		37.5–38.5			(26)
9K7	1-Phenyl-3-methyl-5-ethoxypyrazole	white ndls.	alcohol	236–237			(21)
13K	1-Phenyl-3-methyl-5-chloro-pyrazole methiodide	white ndls.					

[a] The 4-nitrosopyrazoles were dissolved in the solvent with minimum heating at 60° C. or less.

[b] Because of the instability of the 4-nitrosopyrazoles at elevated temperatures, the m.p.'s (or dec.p.'s) of some of them vary markedly with the temperature at which the capillary is introduced into the bath and the rate at which the detn. was begun. The conditions employed for the determination of the recorded m.p.'s (or dec.p.'s) of these compounds are given.

[c] Detn. begun at 145° with a 3°/min. rise in temp.

[d] Detn. begun at 160° with a 4°/min. rise in temp.; beginning at ca. 170° the compound gradually changed from a green to a brown solid, the amt. of decompn. increasing with rise in temp.

[e] Detn. begun at 125° with a 3°/min. rise in temp.; beginning at ca. 189° the compound, with sintering, changed from green to brown, the amt. of decompn. increasing with rise in temp.

[f] Detn. begun at 185° with a 3°/min. rise in temp.

[g] Detn. begun at 160° with a 3°/min. rise in temp.

[h] Obtained from Eastman Kodak Co., "Eastman", grade.

[1] Varies with heating conditions; the recorded m.p. was observed when the detn. was begun at 180° with a 4°/min. rise in temperature. The literature reports m.p.'s varying from 199° to 214° for this compound.

Compound 30J was prepared by the procedure of Wolff *et al.* (31), 39J by the second procedure of Sachs and Alsleben (25), and 4K3 by adding a concentrated aqueous solution of isonitrosoacetylacetone to a cold aqueous solution of methylhydrazine sulfate neutralized with sodium carbonate. Compound 4K5 was prepared as follows: Enanthylacetone (prepared by treating a mixture of ethyl acetate and methyl *n*-hexyl ketone with sodium) was nitrosated in glacial acetic acid by addition of aqueous sodium nitrite. The resulting solution of isonitrosoenanthylacetone was added to an aqueous solution of *p*-chlorophenylhydrazine hydrochloride to give the oily product.

The methods for the preparation of the other compounds, if not the same as those given in the references to a previous synthesis in table 1, are as follows:

Compound J30, by adding aqueous sodium nitrite to a solution of 1-*p*-chlorophenyl-3-methyl-5-pyrazolone in 1:1 hydrochloric acid.

Compound J54, by adding *p*-chlorophenylhydrazine in portions to an equimolar quantity of acetylacetone with cooling. After the addition was completed, the mixture was heated in a boiling-water bath for 20 min.

Compound 14K, by the hydrogenation of J55 in alcohol at room temperature under an initial pressure of 52 lb. of hydrogen with Raney nickel as catalyst.

The solid compounds were purified by recrystallization from the solvents listed in table 1. The 4-nitrosopyrazoles were dissolved in the solvent with minimum heating at 60° C. or less. The moderate temperatures were adopted after J49 was observed to darken when dissolved in boiling alcohol and allowed to crystallize. The dark green product melted 2° lower than the pure compound. Likewise 30J, a dark blue compound when pure, was dark greenish-blue when crystallized from boiling benzene.

Further evidence that the 4-nitrosopyrazoles are unstable at elevated temperatures was obtained when their melting points were determined. Two of them decomposed before showing evidence of melting. Others decomposed while melting. On this account, the rate of heating had to be controlled to obtain reproducible results (Table 1, footnote b).

The stability of 2K5 (m.p. 112°) at 71° C. was determined. There was no change in melting point in 10 days; after 12 days the melting point was 110–111°; after 17 days, 108–111°; after 20 days, 90–95°. The color changed from blue-green to brown during the 20-day period.

The 4-nitrosopyrazoles react readily with dilute sodium hydroxide. Sachs and Alsleben (25) noted that a small amount of sodium hydroxide solution added to a boiling alcohol solution of 1,5-diphenyl-3-methyl-4-nitrosopyrazole changed the solution from green to red-brown. The product was the azoxy compound.

Similar color changes have been observed when aqueous sodium hydroxide was added to an aqueous solution of 4K3 or an alcohol solution of 2K5 at room temperature. The solutions changed slowly from blue and

blue-green, respectively, to brown and could not be restored to the original color by acidification.

<div align="center">EXPERIMENTAL PROCEDURES</div>

A series of standard bioassays was made on all chemicals. The chemicals were suspended or dissolved in distilled water at a concentration of 2000 ppm by using Emulphor EL, a commercial surface-active agent, where necessary and thoroughly agitating in a Waring Blendor. Part of the stock suspension was diluted to 1000 ppm and used for spore germination tests on glass slides by the test tube dilution method recommended by the Committee of The American Phytopathological Society (3). The concentrations specified in the tables refer to initial concentration before diluting 4 volumes with 1 volume of orange juice filtrate and spore suspension. Spores from 7-day-old cultures of *Alternaria oleracca* Milb. (17) and *Sclerotinia americana* (Worm.) Nort. and Ezek. were used for test purposes. Germination records were taken after 18 hours of incubation at 72° F. Sufficient extra spores were counted to provide the equivalent of 100 per cent germination in the untreated controls.

Another aliquot of the chemical suspension was diluted serially in distilled water to provide a dosage series for testing ability to protect tomato foliage against infection by the early blight fungus, *Alternaria solani* (E. and M.) V. and G. A modification of the method described by McCallan and Wellman (18), employing two or three potted 6-in. plants of the variety Bonny Best for each concentration of chemical, was used. The plants were sprayed for 20 sec. at 20 lb. air pressure while being rotated in a spray chamber on a turntable 56 in. from the nozzle of the spray gun. After the spray deposit was thoroughly dry (3 to 16 hr.), the plants and comparable unprotected controls were sprayed with a suspension containing about 20,000 spores of *A. solani* per ml. The DeVilbiss atomizer used discharged 11 ml. during a 20-sec. period of exposure. The plants were held for 19 to 23 hr. at 75° F. and 99 per cent relative humidity to permit spore germination and infection before returning them to the greenhouse. Records were taken 3 to 6 days later on the number of lesions produced on the 15 major leaflets of the three youngest fully expanded leaves. The data were converted to percentage of control on the basis of the average number of lesions on four unprotected plants and plotted on logarithmic-probability paper to obtain an LD95 value. With very few exceptions, a satisfactory linear dosage-response curve was obtained by this procedure.

Seed protectant tests were made in composted soil in the greenhouse and on a well-drained silt loam field, both of which were known to be severely infested with seed-decay and damping-off fungi (*Pythium* spp. and *Rhizoctonia* spp.). Each chemical was applied in dust form at three dosage levels to seed of peas, corn, beets, and spinach. The chemical and seed, each weighed to the nearest 0.01 gm., were placed in a jar, rotated for 30 min.

at 28 r.p.m. on a vertical turntable, and reweighed to determine the actual dosage of chemical adhering to the seed. In greenhouse trials, lots of 25 seeds of corn and peas or 50 seeds of spinach and beets were sown at random in each of eight replications. The seed was covered to a uniform depth, water was added, and the flats held in a chamber at 55° F. and 99 per cent humidity until the seedlings emerged. They were then removed to a greenhouse at 65° to 80° F. where they were held until emergence records were taken about ten days later. Field trials were made with five replications of 100 seeds each for corn, peas, and spinach, and eight replications for beets.

Field spray treatments were applied by conventional power sprayers capable of developing 350 lb. pressure. Apples were sprayed with an orchard gun from the ground at the rate of 10 to 25 gal. per 20-year-old tree, depending upon the size. Tomatoes were sprayed at the rate of about 200 gal. per acre with a portable boom equipped with five nozzles per row. Potatoes were sprayed in the same fashion with a four-nozzle boom. Potatoes of the variety Green Mountain were grown in 20-ft. parallel rows and six single-row replicates were used. Tomatoes of the variety John Baer were grown in a near-by plot, with seven plants per 21-ft. row in a planting spaced 5 by 3 ft. Five single-row replicates of each treatment were used.

In order to have a standard of comparison in all tests, either tetrachloro-p-benzoquinone (Spergon) or 2,3-dichloro-1,4-naphthoquinone (Phygon) was included. These quinones are known to be highly active fungicides capable of satisfactory field performance on seeds and foliage; so anything equivalent or superior to them is considered to have definite practical possibilities.

EXPERIMENTAL RESULTS

Effect of Substitution in the 4-Position

In preliminary tests pyrazoles with a nitroso group substituted in the 4-position were found to be much more active than comparable 4-unsubstituted compounds. A number of compounds were synthesized with nitroso, nitro, amino, phenylazo, or other groups in this position. The ability of six of these compounds to inhibit germination of Alternaria and Sclerotinia spores on glass slides was determined. The results may be summarized briefly as follows. Compound J54 (without substitution in the 4-position and with a p-chlorophenyl group in the 1-position) completely inhibited germination of both types of spores at 1,000 ppm, had no effect on Alternaria spores at lower concentrations, permitted 85 to 89 per cent germination of Sclerotinia at 100 and 10 ppm, and had no effect on Sclerotinia at a dosage of 1 ppm. When the analogous compound (J49) with a nitroso group substituted in the 4-position was tested, both types of spores were completely inhibited at dosages as low as 1 ppm. There was 90 and 99 per cent germination at 0.1 ppm but no effect at lower concentrations.

This observation on the effectiveness of pyrazoles with a 4-nitroso group was further investigated by synthesizing a series of compounds with either

a nitroso (J55), amino (14K), phenylazo (53K), or nitro (L67) group in the 4-position of 1-phenyl-3,5 dimethylpyrazole. The compound with the nitroso group was about as effective as J49 and decisively more effective than any of the others. Compound 53K failed to inhibit spores of either fungus at concentrations of 1000, 100, 10, or 1 ppm. Compound 14K inhibited germination at 1000 ppm but failed at 100, 10, and 1 ppm. Compound L67 with a 4-nitro group was only slightly more active than 14K. These data suggest that the nitroso group has some unusual ability not common to other nitrogen groups when substituted in the 4-position on the pyrazole nucleus, and it is effective irrespective of the type of substituent in the 1-position. The nitroso group accentuates fungitoxicity about a thousandfold.

Substitution of other groups in the 3- and 5-positions had little or no effect. For example, 3-methyl-5-ethoxypyrazole (9K8) inhibited very few spores at 1000 ppm, and 1-phenyl-3-methyl-5-ethoxypyrazole (9K7) did not inhibit germination at concentrations below 10 ppm. 1-Phenyl-3-methyl-5-chloropyrazole (8K3) and its methiodide (13K) were relatively ineffective. The full value of such compounds can be realized only by substitution of a nitroso group in the 4-position. 1-Phenyl-3-methyl-4-nitroso-5-N-methylanilinopyrazole (L63) completely inhibited spore germination at 1 ppm.

Representatives of the above compounds were tested on tomato foliage as protectants against *Alternaria solani*. The weaker compounds were tested over a dosage range of 2000 to 128 ppm and the more active over a range of 256 to 2 ppm. The data on the percentage of disease control are summarized in table 2. For some reason, probably due to the physiological

TABLE 2.—*Effect of varying the substituent in the 4-position of 3,5-dimethylpyrazoles on ability to prevent infection of tomato foliage by Alternaria solani*

Code	Substituent in		Blight control[a] at concentration (ppm)								
	4 position	1 position	2000	1024	512	256	128	64	32	16	8
			Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
J54	Hydrogen	p Chloro phenyl		45.6	38.0		38.1				
J49	Nitroso	p-Chloro- phenyl					100.0	99.9	99.6	93.0	90.4
J55	Nitroso	Phenyl				99.6	99.8	99.3	96.3	89.8	73.2
14K	Amino	Phenyl	92.1	88.1	67.4		36.7				
53K	Phenylazo	Phenyl	85.8	73.2	59.9		43.8				
2,3-Dichloro-1,4-naphthoquinone							99.9	99.6	98.6	92.0	59.9	56.7

[a] Unsprayed plants had an average of 420 lesions.

vigor of the plants, the disease control was definitely superior to that observed in preliminary trials on these same compounds. The relative position of the various materials, however, remained constant; so the data are considered reliable. When the data were plotted on logarithmic-probability paper, very good straight-line dosage-response curves were obtained. The

estimated LD95 values were: J49, 10.4 ppm; J55, 25.5 ppm; 14K, 2300 ± ppm; and 53K, 5000 ± ppm. The higher LD95 values are only rough approximations, since they were obtained by extrapolation so far beyond the available data. Compound J54 caused considerable foliage necrosis at the two higher dosages, and its LD95 value, if any, was something over 10,000 ppm. These data, in general, agree with the observation on spore inhibition recorded above, except that the superiority of J49 over J55 would not have been anticipated from the slide data. All of the materials, except J54, apparently were noninjurious to foliage in this and various other tests made over a two-year period.

Modification of the Pyrazole Nucleus

In view of the high fungitoxic activity of the 4-nitrosopyrazoles, studies were undertaken to determine the effectiveness of closely related compounds such as 49K and 35J:
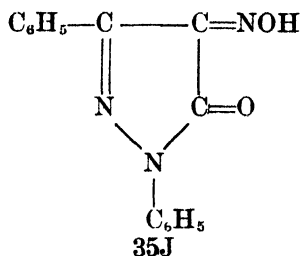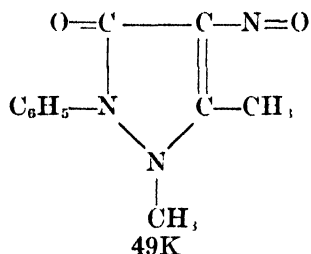
Compound 49K differs from the 4-nitrosopyrazoles in that the 4-nitroso group is attached to a 3-pyrazolone; 35J differs in that the nitroso group has been replaced by an isonitroso group, which is attached to a 5-pyrazolone.

These compounds and J30 were compared to the 4-nitrosopyrazoles J49 and J55 and several related compounds on glass slides and tomato foliage. The data summarized in tables 3 and 4 show J30 and 35J were good fungicides (28) but less effective than the 4-nitrosopyrazoles. Compound 49K was only weakly fungicidal on foliage and had little effect on spores in the glass slide tests.

The tomato foliage data show approximate LD95 values for 49K of 10,000 ± ppm; 35J, 4500 ± ppm; J49, 31.5 ppm; and J55, 60 ppm. In view of these differences, further study was directed toward improving the 4-nitrosopyrazoles by varying the substituents in the 1- and 5-positions rather than toward altering the essential structure of the nucleus.

Effect of Substitution in the 1-Position

The possibility of improving the fungicidal activity of the 4-nitrosopyrazoles by altering the substituent in the 1-position was next investigated. Compounds were synthesized with methyl (4K3), phenyl (J55), and α-naphthyl (19K) groups in the 1-position of 3,5-dimethyl-4-nitrosopyrazole (30J).

TABLE 5.—*Effect of varying the substituents in the 1- and 5-positions of 3-methyl-4-nitrosopyrazole on ability to inhibit germination of fungus spores on glass slides*

Code	Substituent in		Fungus tested	Spore germination[a] at conc. (ppm)			
	1-position	5-position		10	1	0.1	0.01
				Pct.	Pct.	Pct.	Pct.
30J	Hydrogen	Methyl	Alter.	100	100	100	100
30J	Hydrogen	Methyl	Scler.	100	100	100	100
4K3	Methyl	Methyl	Alter.	0	21	100	100
4K3	Methyl	Methyl	Scler.	0	39	100	100
J55	Phenyl	Methyl	Alter.	0	0	15	100
J55	Phenyl	Methyl	Scler.	0	0	97	100
19K	α-Naphthyl	Methyl	Alter.	0	0	100	100
19K	α-Naphthyl	Methyl	Scler.	0	0	98	100
39J	Hydrogen	Phenyl	Alter.	100	100	100	100
39J	Hydrogen	Phenyl	Scler.	10	98	100	100
4K5	p Chlorophenyl	n-Hexyl[b]	Alter.	98	100	100	100
4K5	p Chlorophenyl	n-Hexyl[b]	Scler.	99	100	100	100
Dichloronaphthoquinone			Alter.	0	0	100	100
			Scler.	0	0	54	100

[a] Germination of controls: Alternaria, 96 per cent; Sclerotinia, 91 per cent.
[b] Possibly in 3-position with methyl group at 5.

TABLE 6.—*Effect of varying the substituents in the 1- and 5-positions of 3-methyl-4-nitrosopyrazole on ability to prevent infection of tomato foliage by Alternaria solani*

Code	Substituent in		Blight control[a] at conc. (ppm)								
	1-position	5 position	2000	1024	512	256	128	64	32	16	8
			Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
30J	Hydrogen	Methyl	91.6	91.9	87.9		59.7				
J55	Phenyl	Methyl				99.6	99.8	99.3	96.3	89.8	73.2
19K	α-Naphthyl	Methyl					99.6	100.0	99.3	98.5	92.7
39J	Hydrogen	Phenyl	93.3	85.6	81.8		77.3				
4K5	p-Chlorophenyl	n Hexyl[b]	39.9	78.5	4.0		55.5				
Dichloronaphthoquinone							99.9	99.6	98.6	92 0	59.9	56.7

[a] Unsprayed plants had an average of 420 lesions.
[b] Possibly in 3-position with methyl group at 5.

The records of spore germination show that 30J was ineffective at the low dosages used in this test, while the substitution of a phenyl or α-naphthyl group increased activity at least a hundredfold. The methyl group was not so effective. The substitution of a phenyl group for the methyl group in the 5-position of 30J (see 39J) had very little effect. The phenyl group is most effective when attached to the nitrogen in the 1-position.

The data obtained on tomato foliage substantiate the observations on spore germination on glass slides. When the data on blight control were plotted on logarithmic-probability paper, a satisfactory straight-line dosage

response was obtained. The estimated LD95 values were: 30J, 2500 ± ppm; J55, 25.5 ppm; 19K, 10.4 ppm; 39J, 2500 ± ppm; and 4K5, > 10,000 ppm. There was not sufficient 4K3 available for a foliage test in this series; but in a comparable preceding test it had an LD95 value of 10,000 ppm or slightly less. Hence, it may be considered a weak fungicide.

These studies demonstrate that an aryl group must be substituted in the 1-position if a 4-nitrosopyrazole is to attain maximum effectiveness. Of the two compounds containing aryl groups investigated, the a-naphthyl derivative was more effective. It is, however, more difficult to synthesize than the phenyl derivative; so further studies on modification of the aryl substituent were restricted to the phenyl group.

Effect of Substitution on the 1-Phenyl Group

Since the foregoing studies had shown that 4-nitroso and 1-aryl groups were requisites for maximum fungicidal activity, the next series of studies was devoted to determining the effect of substitution on the 1-phenyl group. The various compounds listed in table 7 were synthesized and tested for ability to inhibit spore germination on glass slides.

The first five compounds listed were chosen in order to determine the effect of the halogens substituted in the *para* position on fungicidal activity. The data on glass slides show that the unsubstituted compound (J55) was the most effective, since spore germination was inhibited by a concentration as low as one part in more than a billion of water. These data are considered somewhat misleading, since in several other tests J55 was approximately equivalent to the p-chloro derivative (J49). The material (J55) is somewhat water-soluble and it is believed that small differences in manipulation of the test affected the results. The p-chloro derivative was about as effective as the p-fluoro derivative, but was approximately ten times more potent than the corresponding bromo and iodo compounds. This observation confirms data obtained in several preceding tests but not reported here.

The shifting of the chlorine to other positions on the phenyl ring materially altered the fungicidal effectiveness. In comparison to the p-chloro derivative (J49), the m-chloro derivative (35L) was only slightly less effective, while the o-chloro compound (27L) was appreciably less active. The two dichloro derivatives (3K3 and 3K4) were only about one-tenth as active as J49, the p-chloro derivative.

These effects of halogen substitutions are of particular interest in view of the important role played by chlorine in the many new organic fungicides and insecticides. The chlorine probably contributes to the blocking of essential functions by p-dichlorobenzene, Chlordane, DDT, metallic trichlorophenolates, chlorinated quinones, *etc*.

In order to determine the effectiveness of other substituents on the 1-phenyl group, phenylhydrazines with methyl, *tert*-amyl, nitro, thiocyano, carboxyl, sulfamyl, and phenyl groups in the *para* position were reacted

TABLE 7.—*Effect of various substituents on the phenyl group in 1-phenyl-3,5-di-methyl-4-nitrosopyrazole on ability to inhibit germination of fungus spores on glass slides*

Compound tested		Test fungus	Germination[a] at concentration (ppm)						
Code	Substituent		100	10	1	0.1	0.01	0.001	0.0001
			Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
J55	None	Alter.	0	0	0	0	0	0	94
J55	None	Scler.	0	0	0	0	32	10	96
0M6	p-Iodo	Alter.	0	0	0	99	98	100	100
0M6	p-Iodo	Scler.	0	0	9	82	91	95	100
7K1	p-Bromo	Alter.	0	0	0	2	100	100	100
7K1	p-Bromo	Scler.	0	0	0	94	100	100	100
4L9	p-Fluoro	Alter.	0	0	0	0	95	100	100
4L9	p-Fluoro	Scler.	0	0	0	1	97	100	100
J49	p-Chloro	Alter.	0	0	0	0	99	97	100
J49	p-Chloro	Scler.	0	0	0	0	90	100	100
35L	m-Chloro	Alter.	0	0	0	0	91	100	100
35L	m-Chloro	Scler.	0	0	0	5	67	88	100
27L	o-Chloro	Alter.	0	0	0	0	96	100	100
27L	o-Chloro	Scler.	0	0	0	60	94	100	100
3K4	2,4 Dichloro	Alter.	0	0	0	100	100	100	100
3K4	2,4-Dichloro	Scler.	0	0	0	100	100	100	100
3K3	2,5-Dichloro	Alter.	0	0	0	97	100	100	100
3K3	2,5-Dichloro	Scler.	0	0	0	96	100	100	100
2K5	p-Methyl	Alter.	0	0	0	0	88	100	100
2K5	p-Methyl	Scler.	0	0	0	0	88	100	100
7K2	p-tert-Amyl	Alter.	0	0	0	100	100	100	100
7K2	p-tert-Amyl	Scler.	0	0	0	86	100	100	100
8K2	p-Nitro	Alter.	0	0	0	81	99	100	100
8K2	p-Nitro	Scler.	0	0	0	93	97	100	100
39L	p-Thiocyano	Alter.	0	0	3	100	100	100	100
39L	p-Thiocyano	Scler.	0	0	0	96	100	100	100
9K5	p-Carboxy	Alter.	0	96	99	97	100	100	100
9K5	p-Carboxy	Scler.	0	93	94	100	100	100	100
36L	p-Sulfamyl	Alter.	100	100	100	100	100	100	100
36L	p-Sulfamyl	Scler.	90	100	100	100	100	100	100
0M9	p-Phenyl	Alter.	0	36	86	100	100	100	100
0M9	p-Phenyl	Scler.	69	95	95	90	96	100	100
Tetrachlorobenzo-quinone		Alter.	0	0	66	98	100	100	100
		Scler.	0	0	63	100	100	100	100

[a] Germination of controls: Alternaria, 96 per cent; Sclerotinia, 92 per cent.

with isonitrosoacetylacetone. The series of 3,5-dimethyl-4-nitrosopyrazoles obtained was tested. Some of these, such as the phenyl (0M9), sulfamyl (36L), and carboxy (9K5) derivatives, were mediocre. The one interesting discovery in this series was that the p-methyl derivative (2K5), i.e., the compound with a p-tolyl radical in the 1-position of the pyrazole ring, was fully as effective as J49 if not superior to it. Apparently the number of carbon atoms in the para position cannot be increased very much if this

extreme activity is to be maintained, since the compound with a *tert*-amyl group (7K2) was less effective.

The full significance of these data can be realized by comparing the performance of these new fungicides to that of tetrachlorobenzoquinone. Only the three least active members were inferior to the standard. The better 4-nitrosopyrazoles were almost 100 times as potent. Spergon has a rating somewhat lower than 2,3-dichloro-1,4-naphthoquinone in glass slide tests, but is equivalent or superior to many other successful fungicides.

Duplicate tests were made on tomato foliage using one series of plants inoculated immediately after spraying and the other, 36 hr. later. Detailed data on the first test and the estimated LD95 values from both tests are presented in table 8. The LD95 values in the second test were consistently

TABLE 8.—*Effect of various substituents on the phenyl group in 1-phenyl-3,5-dimethyl-4-nitrosopyrazole on ability to prevent infection of tomato foliage by Alternaria solani*

| Compound tested | | Blight control[a] at concentration (ppm) in Test 1 | | | | | | | | | LD95 value | |
Code	Substituent	960	480	240	120	60	30	15	7.5	3.8	Test 1	Test 2
		Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	ppm	ppm
J55	None			99.8	99.3	94.4	78.2	74.0			62	120
0M6	p-Iodo			88.4	91.3	77.0	37.6	23.1			170	250
7K1	p-Bromo				81.6	77.5	39.6	17.8	15.9		400	230
4L9	p-Fluoro				100.0	98.8	92.5	68.3	18.1		37	100
J49	p Chloro					97.6	95.2	92.0	42.0	37.0	28	35
35L	m-Chloro					97.8	93.5	89.1	37.4	14.7	34	72
27L	o Chloro				97.1	96.4	92.7	77.3	72.7		60	100
3K4	2,4-Dichloro			99.8	97.6	85.0	43.0	34.5			92	120
3K3	2,5-Dichloro			98.5	97.3	88.4	51.2	47.0			100	120
2K5	p-Methyl					97.8	89.1	93.2	65.2	25.0	30	56
7K2	p-tert-Amyl					86.9	65.4	32.3	12.2	4.3	100	150
8K2	p-Nitro		93.2	87.7	72.0	47.1	50.0				560	900
39L	p-Thiocyano			79.9	82.4	83.6	31.4	5.7			220	750 ±
9K5	p-Carboxy	91.1	55.3	49.5	55.3	72.4					1500	900 ±
36L	p-Sulfamyl		91.8	98.3	79.2	65.7	37.2				560	820
0M9	p-Phenyl	75.1	72.4	68.8	10.5						∞	3500 ±
Dichloronaphthoquinone				100.0	99.3	78.5	25.3	8.9			82	115

[a] Unsprayed plants had an average of 414 lesions.

higher than in the first one, but the relative values were consistent. The compounds fell in about the same relative positions as they did on glass slides. Compounds 2K5 and J49 were superior, with LD95 values of about 30 ppm. The other halogen-containing derivatives had LD95 values of 34 to 400 ppm. Compounds 0M9, 36L, 9K5, 39L, and 8K2 were definitely inferior to the others.

These data on foliage are considered reliable. They are typical of the excellent disease control obtained without visible foliage injury over a testing period of 18 months. In these preceding tests 2K5 had LD95 values of 25 ppm on two occasions, 30 ppm in three tests, and 35 ppm twice. Compound J49 had values of 20 ppm once, 25 ppm six times, 30 ppm four

times, 35 ppm once, and 55 ppm once. These values obtained on plants of different age and vigor agree well with the range of 28 to 56 ppm reported in table 8. Compound J55 did not rate so consistently effective in six preceding tests, since its LD95 values were 55, 80, 140, 145, 150, and 170 ppm. The other compounds listed in table 8 gave disease control in preliminary trials comparable to that reported.

Attention is particularly directed to the performance of 1-p-sulfamyl-phenyl-3,5-dimethyl-4-nitrosopyrazole (36L). The material had negligible ability to inhibit spore germination on glass slides. It was somewhat more effective as a foliage protectant in spite of its poor comparative position in relation to the other compounds. There are several fungicides used commercially that would do no better in this particular test. These data, however, offer no suggestion that this might be an exceptionally effective fungicide on seeds, as will be shown in table 11.

These studies on the substituents on the 1-phenyl group climaxed the efforts to create a superior fungicide of the 4-nitrosopyrazole class. The success of this effort may be realized by making comparisons with the two quinone fungicides used as standards. Thirteen of the 16 compounds reported in table 7 were more toxic to spores on glass slides than was tetrachlorobenzoquinone (Spergon), a widely used commercial product. In the foliage tests there were seven compounds that were fully as effective as 2,3-dichloro-1,4-naphthoquinone (Phygon), an outstanding fungicide that is finding many practical applications (4, 5, 6, 9, 23).

The outstanding representatives of this class are 1-p-tolyl-3,5-dimethyl-4-nitrosopyrazole (2K5) and 1-p-chlorophenyl-3,5-dimethyl-4-nitrosopyrazole (J49). These nitrosopyrazoles were almost 100 times as active as tetrachlorobenzoquinone on slides and two to three times as effective as 2,3-dichloro-1,4-naphthoquinone on tomato foliage. No other group of nonmercurial organic compounds has been reported as possessing comparable fungitoxicity. If materials with this activity persist on foliage under field conditions, adequate disease control might be expected from a dosage of about 4 oz. or less per 100 gal. of spray mixture, since 12 to 16 oz. of Phygon will provide excellent control of apple scab and tomato leaf blight diseases.

Fungitoxicity of Reactants Used to Produce 4-Nitrosopyrazoles

Inasmuch as 4-nitrosopyrazoles are produced spontaneously in aqueous medium from a hydrazine (generally as the hydrochloride) and isonitrosoacetylacetone, it would seem unlikely that they could be hydrolyzed to regenerate the reactants. However, the possibility exists that the fungus could act as a hydrolytic agent and that one or both of the reactants account for the fungitoxicity of the 4-nitrosopyrazoles. The theory was sufficiently interesting to warrant testing; so an experiment was arranged wherein the compounds used to produce the 4-nitrosopyrazoles were tested alongside the appropriate reaction product.

Although many hydrazines were tested in the course of these studies, none of them were more fungitoxic than phenylhydrazine (E24); so it was chosen for these tests. When reacted with isonitrosoacetylacetone (P90), compound J55 is produced. Other hydrazines, α-naphthylhydrazine (16K) and p-chlorophenylhydrazine hydrochloride (5J0), and the nitrosopyrazoles (19K and J49) produced by reacting them with isonitrosoacetylacetone, were also included in these tests. There was not sufficient α-naphthylhydrazine available for the foliage tests in this series, but in previous tests it had proved to be relatively ineffective as a foliage protectant (87 per cent control at 2000 ppm and an LD95 > 10,000 ppm).

Isonitrosoacetylacetone was practically nonfungitoxic even at dosages as heavy as 1000 ppm on glass slides and 2000 ppm on foliage (tables 3 and 4). Phenylhydrazine was fairly active on glass slides but was ineffective on tomato foliage. It was only a fraction as active in either test as the nitroso-pyrazole (J55) produced by reacting it with isonitrosoacetylacetone.

The nitrosopyrazoles produced from α-naphthylhydrazine and p-chloro-phenylhydrazine were 100 to 1000 times as effective as the parent hydrazine on glass slides. The data from tomato foliage tests provide an even better contrast. The three nitrosopyrazoles had LD95 values of 60 ppm (J55), 31.5 ppm (J49), and 30 ppm (19K). The two hydrazines used in synthe-sizing J55 and J49, respectively, had LD95 values of approximately 10,000 ppm (E24) and 3000 ppm (5J0).

The obvious conclusion from this test is that if the fungus or plant did hydrolyze the 4-nitrosopyrazole, the hydrolysis products would not be very fungicidal. The nitrosopyrazole must act as an entity. This is a logical assumption since they are relatively stable.

Chemotherapy and Solubility Experiments

The tomato foliage protection test is based upon the assumption that a uniform layer of fungitoxic chemical is deposited in position to prevent spore germination. Most of the good fungicides must be applied at concentrations of 200 to 600 ppm in order to provide 95 per cent control; so comparable control by the better 4-nitrosopyrazoles at concentrations of 10 to 60 ppm raises a question as to whether they operate in a conventional manner. They conceivably may operate in a molecular rather than particulate state as chemotherapeutic agents inside the leaves or as solutes on the leaf surface. Several of the more active compounds reported upon in table 8 were suspended in distilled water at 2000 ppm, employing Emulphor EL where necessary, and tested for solubility and chemotherapeutic activity by bioassay on tomato foliage.

Tests were made in triplicate on potted 7-in. plants. Three 30-ml. vials were filled with a 24-hr.-old suspension of each chemical, and the first fully expanded leaf on each plant was inserted in a vial. The vials were sealed with moisture-proof paper to prevent the chemical from reaching the sur-

face of adjacent leaves by splashing or volatilization. The plants were
held in a greenhouse at 65° to 80° F. without watering for 36 hr. to permit
uptake of chemical by the immersed leaf. About 8 ml. of suspension was
absorbed before the leaf petiole was cut off and the retaining vial removed.
The plants were watered, sprayed with a spore suspension of *Alternaria
solani,* and placed in an incubation chamber for 24 hr. They were then
removed to the greenhouse for 72 hr. before records were taken on the
number of lesions.

The data in column 3 of table 9 show that J55 gave 44.4 per cent control

TABLE 9.—*Chemotherapeutic activity of several 1-(substituted phenyl)-3,5-dimethyl-
5 nitrosopyrazoles*

Compound tested		Leaf blight chemotherapy	Control by filtrate spray	Estimated concentration in filtrate
Code	Substituent			
		Pct.	*Pct.*	*Ppm*
J55	None	44.4	88.6	37
0M6	*p* Iodo	7.7	58.1	38
7K1	*p*-Bromo	21.3	63.7	49
4L9	*p*-Fluoro	22.6	88.1	27
J49	*p* Chloro	24.0	32.7	5
35L	*m*-Chloro	11.6	75.8	12
27L	*o* Chloro	18.8	71.7	9
3K4	2,4-Dichloro	24.4	87.2	61
3K3	2,5 Dichloro	24.3	87.4	60
2K5	*p* Methyl	19.5	26.2	4
7K2	*p tert* Amyl	2.2	89.3	69
8K2	*p* Nitro	5 5	84.0	210
39L	*p*-Thiocyano	5.4	66.6	76
36L	*p* Sulfamyl	31.0	94.7	515
Min. Sign. Diff. at 5 per cent		9.6		
Min. Sign. Diff. at 1 per cent		17.0		

and 36L, 31 per cent; hence it must be deduced that some of the chemical
was taken up by the immersed leaf and transported to the other leaves,
where it provided appreciable protection by chemotherapy. Compound 36L
was observed to be particularly effective in delaying the development of
lesions. Counts made 40 hr. after inoculation showed 10 to 30 lesions per
plant, while untreated plants had 250 to 350 lesions each. The relatively
weak fungicides 7K2, 8K2, and 39L gave no significant control of blight.

These data on chemotherapy were not in all cases in close agreement
with those from protectant tests (Table 8). The most obvious deviation was
the unexpected superiority of 36L over J49, 2K5, and other superior pro-
tectants. These apparent inconsistencies were resolved by studies on the
solubilities of the compounds. A 72-hr.-old suspension at a concentration
of 2000 ppm was filtered through Whatman No. 5 paper, then passed
through a fine sintered-glass filter. The filtrates probably contained some
colloidal material in addition to the dissolved fraction, but a fair measure
was obtained of the dissolved and fine fractions that could be easily drawn
into the plant and circulated through its conductive tissue.

These filtrates were sprayed on duplicate plants, and data on blight control were obtained by the conventional method. The data in the fourth column of table 9 show 26 to 95 per cent disease control. Where these percentages were interpolated on the dosage-response curves obtained on comparable plants at the same time (Table 8), estimates were obtained of the actual concentrations of active ingredients. These estimates, presented in the last column of table 9, show that the concentrations of the most highly fungitoxic compounds in the filtrates probably were not greater than 4 ppm for 2K5, 5 ppm for J49, 9 ppm for 27L, and 12 ppm for 35L. Compound 36L, which is not exceptionally fungitoxic, probably achieved its excellent chemotherapeutic performance because of its greater solubility. Compound J55, which excelled 36L in chemotherapeutic protection, is much less soluble, but the dissolved material probably is about eight times as potent (Table 8) and hence much more effective at low concentrations. On the other hand, J55 is so much more soluble than the superior fungicides that it has an opportunity to be taken into the plant in sufficient quantity to provide systemic protection.

These tests show that J49 and 2K5 have the characteristics essential for a good foliage protectant. They combine strong fungitoxicity with minimum solubility; so they should be retained on the foliage at effective concentrations during rainstorms. Because of this, these two compounds were used for extensive field trials as seed and foliage protectants.

Value of 4-Nitrosopyrazoles as Pea Seed Protectants

Several nitrosopyrazoles were tested for ability to protect Thos. Laxton and Perfection pea seed in the greenhouse and field. The effect of various substituents in the 1-position of 3,5-dimethyl-4-nitrosopyrazole was determined in two tests made in the greenhouse on different dates with the seven compounds listed in table 10. Seed treated with each chemical at three dosage levels was sown in naturally infested soil. In the first test the soil was moderately moist, while in the second it was watered heavily so that seed decay would be more severe. Spergon was included in the test as a standard, since it is widely used on pea seed.

The only injurious material was the soluble 1,3,5-trimethyl-4-nitrosopyrazole (4K3) which gave significant improvement in emergence at the extremely low dosage of 0.02 per cent but suppressed germination at higher dosages. Compounds 7K2 and 3K4 were somewhat less effective than the other nitrosopyrazoles, a situation anticipated from their fungitoxicity records. The other compounds, particularly J55, J49, and 2K5, were fully as effective as Spergon at comparable dosages. In general, the seed protectant ability of these compounds agreed closely with their fungitoxicity as shown in tables 7 and 8.

Comparable confirmatory data were obtained on 0M6, 4L9, and L67 in another greenhouse test made on Thos. Laxton pea seed. The mean emer-

TABLE 10.—*Effectiveness of several 3,5-dimethyl-4-nitrosopyrazoles with various substituents in the 1-position as pea seed protectants in severely infested greenhouse soil*

Treatment applied to seed			Emergence of plants	
Code	Substituent	Dose	Test 1	Test 2
		Pct.	*Pct.*	*Pct.*
J49	*p*-Chlorophenyl	0.46	82.0	66.0
J49	*p*-Chlorophenyl	0.09	71.0	54.5
J49	*p*-Chlorophenyl	0.02	70.0	47.5
J55	Phenyl	0.55	82.5	73.5
J55	Phenyl	0.13	80.5	58.5
J55	Phenyl	0.03	71.0	51.0
7K2	*p-tert*-Amylphenyl	0.54	70.0	47.0
7K2	*p-tert*-Amylphenyl	0.13	63.5	45.0
7K2	*p-tert*-Amylphenyl	0.02	64.5	45.0
7K1	*p*-Bromophenyl	1.05	73.5	61.5
7K1	*p*-Bromophenyl	0.25	70.0	63.0
7K1	*p*-Bromophenyl	0.10	68.0	50.5
2K5	*p*-Tolyl	0.51	81.0	64.5
2K5	*p* Tolyl	0.14	75.5	61.0
2K5	*p*-Tolyl	0.04	72.0	51.5
3K4	2,4-Dichlorophenyl	0.59	63.5	61.0
3K4	2,4-Dichlorophenyl	0 09	62.5	49.0
3K4	2,4-Dichlorophenyl	0.01	64.0	44.0
4K3	Methyl	0.28	39.0	22.5
4K3	Methyl	0.17	64.5	40.0
4K3	Methyl	0.02	73.5	52.0
Spergon		0.78	80.0	62.5
Spergon		0.16	77.0	56.0
Spergon		0.01	71.0	47.5
None			59.5	35.5
None			66.5	33.5
None			62.5	37.5
None			61.5	30.5
Min. Sign. Diff. at 5 per cent			9.9	11.6
Min. Sign. Diff. at 1 per cent			13.1	15.2

gence in eight replications of 25 seeds each was: 14 and 23.5 per cent in the untreated controls; 87.5, 83.5, and 68 per cent for Spergon-treated seed at 0.43, 0.20, and 0.10 per cent by weight; 80.5, 70.0, and 48 per cent for 4L9 (1-*p*-fluorophenyl-3,5-dimethyl-4-nitrosopyrazole) at 0.17, 0.10, and 0.03 per cent; 67.5, 60.5, and 57.5 per cent for 0M6 (1-*p*-iodophenyl-3,5-dimethyl-4-nitrosopyrazole) at 0.14, 0.03, and 0.01 per cent. These data show the 1-*p*-fluorophenyl derivative to be as effective as Spergon and superior to the analogous 1-*p*-iodophenyl compound. In this test seed treated with L67 (1-phenyl-3,5-dimethyl-4-nitropyrazole) produced 39, 35, and 30 per cent emergence at dosages of 0.20, 0.09, and 0.04 per cent by weight. The results were to be anticipated from the relatively weak fungitoxicity exhibited by this compound both on glass slides and tomato foliage.

Another greenhouse test was made with pea seed of the variety Thos. Laxton to determine the relative effectiveness of compounds containing different substituents on the 1-phenyl group. The data presented on 27L, 35L, 36L, 39L, and Spergon in table 11 show all of these materials to be excel-

TABLE 11.—*Relative effectiveness of 3,5-dimethyl-4-nitrosopyrazoles as pea seed protectants under greenhouse conditions*

Treatment applied		Emergence from seed with dose of		
Code	Substituent in 1-position	0.20 per cent	0.10 per cent	0.05 per cent
		Pct.	*Pct.*	*Pct.*
27L	*o*-Chlorophenyl	68.0	66.0	49.0
35L	*m*-Chlorophenyl	68.5	57.0	52.5
36L	*p*-Sulfamylphenyl	85.5	76.0	60.5
39L	*p*-Thiocyanophenyl	65.5	58.5	49.0
Spergon		77.0	59.0	57.5
None		34.0	38.5	
Min. Sign. Diff. at 5 per cent				18.0
Min. Sign. Diff. at 1 per cent				24.0

lent seed protectants. The outstanding member is 36L (1-*p*-sulfamylphenyl-3,5-dimethyl-4-nitrosopyrazole), a compound derived from sulfanilamide. It is surprising that this material proved to be fully as effective as Spergon since it was not outstanding on glass slides (Table 7). The question may be raised as to whether its chemotherapeutic activity and water solubility (Table 9) may not have given it an advantage in this trial.

The effectiveness of 36L was amply confirmed in a field trial. The mean emergence in five replications of 100 seeds each treated with 36L at dosages of 0.30, 0.15, and 0.07 per cent by weight was 86.2, 89.3, and 87.8 per cent, respectively, as compared to 88.8, 90.5, and 79.7 per cent for Spergon at comparable dosages, and 69.2, 68.8, and 71.0 per cent for untreated controls. In this test the minimum significant difference at the 5 per cent point was 8 per cent; at the 1 per cent point, 11 per cent. These data were confirmed in 1947 and 1948 by other tests made in the greenhouse and field on seeds of peas, beets, and sweet corn.

Some of the compounds evaluated under greenhouse conditions were given more extensive trials in one greenhouse and two field plantings made during the 1946 season. The compounds used had different substituents in the 1-position of 3,5-dimethyl-4-nitrosopyrazole. All materials except 2K5 which was diluted in talc were applied as concentrated dusts. Each formulation was applied at dosages of approximately 0.64, 0.16, and 0.04 per cent by weight of the pea seed. The mean emergence obtained from 5 replications of 100 seeds each in the first field trial at each of these dosages was as follows: 2K5 (*p*-tolyl derivative) at 100 per cent concentration 79.8, 86.4, and 79.4; 2K5 at 50 per cent concentration 82.0, 72.6, 74.8; 2K5 at 25 per cent concentration 76.4, 84.0, 68.2; J49 (*p*-chlorophenyl derivative) 82.0, 82.8, 80.4; J55 (phenyl derivative) 75.8, 68.2, 77.8; 7K1 (*p*-bromophenyl derivative) 85.2, 89.0, 78.0; 7K2 (*p-tert*-amylphenyl derivative) 84.2, 67.6, 72.6; tetrachloro-*p*-benzoquinone and DDT 85.6, 83.0, 75.4; and untreated controls 74.0, 68.0, and 78.8 per cent, respectively. The minimum significant differences at the 5 and 1 per cent points were 13.6 and 17.9 per cent, respectively.

In the second field trial the mean emergence from seed bearing the same dosages of the materials as listed in the above order was: 89.6, 86.4, and 80.8; 81.2, 81.0, and 76.8; 82.4, 76.8, and 74.4; 88.4, 81.0, and 81.0; 84.8, 80.6, and 76.2; 88.0, 84.4, and 79.8; 82.0, 78.6, and 73.4; 86.0, 86.0, and 80.5; and 71.6, 70.0, and 69.0. The minimum significant difference between means was 6.4 at the 5 per cent level and 8.4 at the 1 per cent level.

Slightly more severe conditions prevailed in the trial made in the greenhouse, but comparable results were obtained. The corresponding emergence from seed treated with the materials listed above was: 89.6, 88.0, and 72.0; 87.2, 80.0, and 68.0; 83.2, 77.6, and 54.4; 80.8, 88.0, and 80.0; 88.0, 90.4, and 76.8; 80.8, 86.4, and 70.4; 68.8, 64.8, and 60.8; 88.0, 87.2, and 70.4; and 61.6, 60.8, and 62.4, respectively. The minimum significant differences between means in this test were 5.7 and 7.6 per cent at the 5 and 1 per cent levels.

The data from these three experiments are in close agreement. The better nitrosopyrazoles compared favorably with Spergon. Compound J55 did not prove superior either to 2K5 or J49; so it is concluded that the small differences in its favor in preceding tests (Table 10) were entirely attributable to experimental error. Compound 7K2 continued to provide poor seed protection under conditions favorable to seed decay. When 2K5 was diluted with talc, its effectiveness was reduced. It may be concluded that the material is approximately twice as effective as Spergon, since the 50 per cent concentration gave results analogous to those of Spergon at full strength.

The Effectiveness of Two 4-Nitrosopyrazoles as Seed Protectants for Corn, Spinach, and Beets

Two of the effective pea seed protectants, J49 and 2K5, were tested in field trials as protectants for sweet corn seed of the variety Golden Cross Bantam (Table 12). Two experiments were made employing five replications of 100 seeds each from each dosage. In the first test, repeated heavy rains and 10 cold days immediately after planting created ideal conditions for seed decay. Only 33 per cent of the untreated seed produced plants and these were small and weak. Over 73 per cent of the Phygon-treated seed produced plants. Under the conditions of this experiment this material would equal or surpass other commercial products. Compound 2K5 was about as effective as Phygon at the two higher dosages but did not give comparable protection at the lowest dosage. However, its performance would definitely classify it as a commercial possibility.

Compound J49 was less effective than 2K5 at all dosage levels. However, it was definitely a better protectant than Semesan Jr. which was used as a standard of comparison. Under the more moderate disease conditions prevailing in the second test, J49 was as satisfactory as the other two materials. This test was not sufficiently severe to provide a true measure of fungicidal efficiency, since there was little difference among either the materials or the various dosages.

TABLE 12.—*Relative effectiveness of two 4-nitrosopyrazoles and standard fungicides as sweet corn seed protectants under field conditions*

Treatment applied		Seedling emergence	
Material	Dose	Test 1	Test 2
	Pct.	Pct.	Pct.
Phygon	0.54	73.2	67.4
Phygon	0.20	74 0	70.6
Phygon	0.07	72.8	65.6
2K5	0 52	72.4	73.4
2K5	0.18	70.0	71.2
2K5	0.07	54.0	69.4
J49	0.54	64.6	74.0
J49	0.19	61.2	69.2
J49	0.07	47.4	70.6
Semesan Jr.	0.19	45 0	71.0
None		32 2	64.4
None		33.4	65.0
None		33.0	64.2
Min. Sign. Diff. at 5 per cent		7.3	5.6
Min. Sign. Diff. at 1 per cent		9.7	7.5

The same two nitrosopyrazoles were tested on spinach seed of the variety Virginia Savoy at three dosage levels in a series of greenhouse and field trials (Table 13). Phygon and Phygon-DDT were used as standards

TABLE 13.—*Relative effectiveness of nitrosopyrazoles and standard fungicides as spinach and beet seed protectants under field and greenhouse conditions*

Treatment applied to seed		Emergence from 100 seed units			
		Spinach		Beets	
Material	Dose	Field	G.H.	Field	G.H.
	Pct	Pct.	Pct.	Pct.	Pct.
Phygon	2.00	60 6	78 0	58.3	110.5
Phygon	1 00	66 6	81.6	58.8	107.8
Phygon	0 50	68 4	76 0	71.9	100.2
Phygon DDT	2.00	69.0	79 6	65 8	100.2
Phygon DDT	1.00	65 8	80.4	71.9	99.2
Phygon-DDT	0.50	61.2	81.2	68.8	95.2
2K5 (100 per cent)	2.00	63.0	74.0	63 5	99.5
2K5 (100 per cent)	1.00	61.4	76 0	63.5	105.0
2K5 (100 per cent)	0 50	53 0	74.4	61.5	81.8
2K5 (25 per cent)	2.00	56.0	78.0	66 0	84.0
2K5 (25 per cent)	1.00	59 4	73.2	54.3	69.5
2K5 (25 per cent)	0.50	45 6	76.4	42.5	55.8
J49 (100 per cent)	2.00	47.2	72.4	71 0	100.8
J49 (100 per cent)	1.00	52.4	78.4	66.5	72.5
J49 (100 per cent)	0.50	40.4	66.4	54.3	54.5
None		23.2	27.6	13 0	9.2
None		22.8	25.6	15.3	9.2
None		24.0	23.6	18.1	7.8
Min. Sign. Diff. at 5 per cent		9.2	8.7	7.3	24.0
Min. Sign. Diff. at 1 per cent		12 2	11.5	9.6	31.8

since they were known to be exceptionally effective on this crop. The data obtained agree fairly well with those obtained on corn seed. Under field conditions the 2,3-dichloro-1,4-naphthoquinone formulations were slightly more effective than 2K5 at the lowest dosage but not significantly different at the higher dosages. The initial stand obtained in the greenhouse showed 2K5 to be equal to Phygon when used at 100 per cent concentration. When 2K5 was diluted to 25 per cent with talc, it performed as well as the other materials in the greenhouse but was less effective under field conditions. Compound J49 was slightly (but not significantly) less effective than 2K5 under greenhouse conditions and was definitely less effective in the field trial.

Tests similar to those on spinach were made on beet seed of the variety Asgrow Canner (Table 13). The average emergence in eight replications of 100 seed balls each in the first field trial showed that 2K5 was slightly more effective (but not significantly so) than Phygon. In a second field trial there was no appreciable difference between the two treatments at the higher dosages, but Phygon was superior at the lowest dosage employed. Under greenhouse conditions the materials again gave approximately the same results. Compound 2K5 was also very effective when used as a 25 per cent dust in talc at dosages of 1 to 2 per cent by weight of seed.

Compound J49 provided good seed protection when used at a dosage of 2 per cent. At lower dosages it gave mediocre protection. It should be noted that all of these tests were under conditions extremely favorable to seed decay, and even the least effective dosage improved emergence by 50 to 300 per cent.

Value as Foliage Protectants

On the basis of their ability to protect tomato foliage in the greenhouse, J49 and 2K5 were selected as the most promising of the nitrosopyrazoles for testing as foliage protectants in field trials. In addition to being active fungicides, they were relatively insoluble and without injurious effects when sprayed on tomato foliage at concentrations as high as 3000 ppm. The practical dosage was estimated to be 4 oz. or less per 100 gal. of spray; so a dosage series of 8, 4, 2, and 1 oz. was selected for apple spray tests.

The treatments were applied at these four dosages to single-tree replicates on the varieties McIntosh, Rome Beauty, Northern Spy, and Baldwin in an orchard about 20 years of age. The 1946 season was extremely favorable to the development of scab (*Venturia inaequalis* (Cke.) Aderh.) and rust (*Gymnosporangium juniperi-virginianae* Schw.). Two prebloom, a calyx, and four cover sprays were applied (April 22, May 1 and 20, June 3 and 17, July 2 and 26). During the 19-day interval preceding the calyx spray a series of five heavy rains and continuous cloudy weather for a week created ideal conditions for spread of conidia produced from primary infection. The schedule followed was obviously inadequate for such a season, and the less effective materials permitted serious foliage infection.

Records were taken on June 26 of scab infection on leaves at six loca-

tions on each tree at 4 to 7 ft. above the ground level. The data on the variety McIntosh presented in table 14 show that J49 provided as good protec-

TABLE 14.—*Protection of apple fruit and foliage from scab and rust by J49 and other fungicides—1946*

Spray treatment		Amount of scab infection			Cedar rust	
Material applied	Dose/100 gal.	On leaves June 26	Lesions per fruit	Commercial control	Lesions per leaf	Disease control
	Oz.	Pct.	No.	Pct.	No.	Pct.
Phygon, wet.	32	5	0.00	100.0	0.097	93.6
Phygon, wet.	16	2	0.80	89.6	0.357	76.5
Phygon, wet.	8	3	0.25	98.8	0.462	69.5
Phygon-Sulfur[a]	32	1	0.02	100.0	0.429	71.7
Phygon-Sulfur[a]	16	6	0.01	100.0	0.443	70.8
Phygon-Sulfur[a]	8	14	0.51	99.7	0.800	47.2
J49	8	6	0.10	99.7	1.386	8.6
J49	4	5	0.29	99.3	1.110	26.8
J49	2	44	46.30	39.6	1.670	0.0
J49	1	29	44.06	33.4	1.286	15.2
Lime-Sulfur	(1.5 gal.)	6	0.11	93.9	0.719	52.6
Wettable Sulfur	128	70	7.29	74.3		
None		75	23.47	44.9	1.516	

[a] Phygon through calyx; wettable sulfur 8 lb. per 100 gal. in cover sprays.

tion at dosages of 8 and 4 oz. as lime-sulfur at 1.5 gal. per 100 gal., and was only slightly less effective than Phygon at the higher dosages. It was decisively superior to wettable sulfur. There was a sharp break in disease control by J49 between dosages of 4 and 2 oz. per 100 gal. Comparable data were obtained on foliage protection of Rome Beauty and Northern Spy.

As the fruit was harvested from each tree it was classified according to the presence of commercial scab; and 2 bushels of fruit, one from the upper branches and the other from the lower ones, were examined for total number of lesions. The data in table 14 show that 45 per cent of the unsprayed fruit escaped severe infection but that there was an average of 23.47 lesions per fruit. The data on fruit infection followed the trend observed on foliage with J49 providing remarkable control at dosages of 8 and 4 oz. but failing at lower rates.

The data in table 14 should be taken as a strong indication of relative merits rather than as complete proof because of the lack of replication of each dosage on the various varieties. The data demonstrated, however, that J49 used at a dosage of 4 oz. per 100 gal. provided as good scab control as lime-sulfur at 1.5 gal. The large number of lesions on fruit sprayed with J49 at low dosages is unexplained. In the latter part of July, many small superficial scab lesions developed uniformly on these trees. These multiplied in number even though few of them caused large lesions or cracking of the fruit.

The orchard was bordered on two sides by natural vegetation which included many rust-infected red cedars. The variety Rome Beauty became

severely infected with rust during the first two weeks of May. At the time the foliage was being examined for scab, the number of rust lesions on this variety was counted. The data summarized in the last two columns of table 14 leave little doubt that J49 was as weak against rust as it was effective against scab. It failed to equal lime-sulfur in rust control. Phygon was definitely superior to both materials.

There was no evidence of foliage injury from J49 at these dosages or at the rate of 1 lb. per 100 gal. applied to three trees outside this experiment. The fruits of McIntosh, Rome Beauty, and Northern Spy were not injured, but Baldwin fruit had appreciable russet at dosages as low as 2 oz. per 100 gal.

Compounds J49 and 2K5 were applied in dosage series to single-row plots of John Baer tomatoes and Green Mountain potatoes. The materials were applied on July 30 and August 9, 22, and 30. This schedule was undoubtedly too abbreviated for the 1946 season. At least one more application could have been used to advantage on tomatoes and three more on potatoes. The season was cool and frequent rains encouraged the development of *Phytophthora infestans* (Mont.) deB. The disease was very prevalent on unsprayed plants by August 15 and most of the foliage was dead by September 15. Severe fruit rot was observed on the tomatoes by the latter part of August, and practically no marketable fruit was harvested from the controls after the first picking.

Both of the nitrosopyrazoles provided considerable protection, as can be seen from the records on blight control in table 15, taken by the visual scor-

TABLE 15.—*Effectiveness of nitrosopyrazoles in preventing Phytophthora blight of tomato and potato*

Spray treatment		Tomato trials		Potato trials	
Material applied	Dose/100 gal.	Blight control	Marketable fruit[a]	Blight control	Yield of tubers[b]
	Oz.	Pct.	Lb.	Pct.	Lb.
2K5	16			64	18.6
2K5	8	84	12.83	82	17.4
2K5	4	80	10.73	64	12.9
2K5	2	65	10.49	25	11.6
J49	16			79	16.9
J49	8	76	10.67	58	13.7
J49	4	77	7.95	43	14.3
J49	2	69	9.35	50	11.6
Phygon, wet.	32	94	43.49	94	24.3
Phygon, wet.	16	92	32.82	93	18.9
Phygon, wet.	8	76	20.13	85	19.4
Phygon, wet.	4			81	16.1
None		35	7.4	9	13.3
Min. Sign. Diff. at 5 per cent			6.67		6.3
Min. Sign. Diff. at 1 per cent			8.86		8.3

[a] Yield from 7 plants.
[b] Yield from 25 plants.

ing method. The control was not considered as commercially acceptable and was definitely inferior to that obtained from Phygon. These differences were accentuated later in the season, and the plants sprayed with the nitrosopyrazoles were practically defoliated by late September. This failure to control blight was reflected in the yields of both crops. There was a moderate increase from their use but it was not so large as that obtained from Phygon.

The reason for this partial failure of the nitrosopyrazoles is not immediately clear. The dosage level may have been too low to control the disease in a year of extremely severe infestation, or the reduced spray schedule may have been responsible. It is also possible that this type of fungicide is ineffective against *Phytophthora infestans* in the same fashion as it failed against cedar-apple rust and as certain other fungicides such as sulfur and metallic dithiocarbamates fail to control late blight. This latter possibility is considered to be the most probable explanation.

Dermatological Effects

The various nitrosopyrazoles were used throughout the series of laboratory and greenhouse tests without observing any adverse effects on the experimenters. In conducting orchard spray tests with J49 and 2K5, the operator noticed a moderate irritation immediately beneath his eyes but it was not considered serious. Appreciable irritation did occur in one test when the sleeve of the operator's coveralls became saturated with spray. His wrist was sensitized for about 10 hr. at the point where the sleeve rubbed against it.

Although the materials did not cause serious dermatitis to the spray operators, they did cause a rash on factory operators who dried and ground large samples for field evaluation. It was considered desirable, therefore, to conduct dermatological tests on 2K5, J49, and other pyrazoles. Thirteen compounds were chosen for tests on white rabbits. Undiluted chemical was rubbed into an area behind the shoulders that had been shaved two days previously. The material was sealed into place by covering it with lanolin. This is an unusually severe method of testing but it has the advantage of detecting weakly irritant materials.

The data in table 16 show that the best fungicides (2K5 and J49) were among the more irritant and the relatively poor fungicide 7K2 (with a *p-tert*-amylphenyl group in the 1-position) was relatively nonirritant. With other substituents in the *para* position of the phenyl group, irritant effects between these extremes were observed. The fluorine derivative was the safest of the halogen-containing compounds. The compound with an unsubstituted phenyl group (J55) was one of the more irritant. The replacement of the phenyl group in the 1-position by a hydrogen atom appreciably reduced the irritant effect but did not completely eliminate it.

Since the nitroso group is generally recognized as causing dermatological

TABLE 16.—*Dermatological effects on rabbits of 3,5-dimethylpyrazoles containing various substituents in the 1- and 4-positions*

Code	Substituent in		Condition of skin after	
	1-position	4-position	24 hours	168 hours[a]
J49	p-Chlorophenyl	Nitroso	Intense red, swollen	Dry, red, sloughing
2K5	p Tolyl	Nitroso	Intense red, swollen	Red, swollen
J55	Phenyl	Nitroso	Red, swollen	Very red, sloughing
4K3	Methyl	Nitroso	Intense red	Red, swollen
8K2	p-Nitrophenyl	Nitroso	Red, swollen, sensitive	Red, swollen
7K1	p-Bromophenyl	Nitroso	Red, swollen, sensitive	Red, sloughing
19K	α-Naphthyl	Nitroso	Red, not sensitive	Red
30J	Hydrogen	Nitroso	Slight red, not sensitive	Slight red
J54	p-Chlorophenyl	Hydrogen	Red, swollen, sensitive	Normal, hair growing
7K2	p-tert-Amylphenyl	Nitroso	Red, not sensitive	Normal
4L9	p-Fluorophenyl	Nitroso	Red, not sensitive	Normal, hair growing
3K3	2,5-Dichlorophenyl	Nitroso	Normal	Normal, hair growing
3K4	2,4-Dichlorophenyl	Nitroso	Trace of red	Normal, hair growing
Lanolin control			Normal	Normal

[a] None of the treated areas seemed sensitive but some edema was evident in treatments 4K3, 2K5, and 8K2.

effects, compound J54, with a hydrogen atom instead of a nitroso group in the 4-position, was included in the test. This material had preliminary irritant properties comparable to the nitroso derivative, but the animal recovered more rapidly.

Of particular interest was the mild reaction incited by 3K3 and 3K4, the 1-(dichlorophenyl) derivatives. These materials, which are excellent fungicides, caused much less dermatitis than any of the other nitrosopyrazoles in this test. They were next subjected to patch tests in undiluted form on the forearm of humans. Some individuals showed very little reaction, but others had pronounced reddening and swelling beneath the patch.

In general, these compounds show dermatological effects in proportion to their fungicidal activity. There are enough exceptions to this rule, however, to offer some encouragement. The fact that such good fungicides as 3K3, 3K4, and 19K are moderately safe provides room for development of safe, effective fungicides.

In another test made on rabbits, 36L, after its ability to protect seed had stimulated interest in it, was found to be nonirritant. There was no swelling or reddening after 2 days and the hair was growing back normally on the 8th day after treatment.

It may be concluded that there is a tendency for the 4-nitrosopyrazoles to irritate when maintained in intimate contact with the skin for prolonged

periods. It is possible, however, to modify the substituent in the 1-position so as to eliminate much, if not all, of this irritant effect.

DISCUSSION

Most of the compounds discussed in this paper are related to 3,5-dimethylpyrazole (1E1):

CH₃—C——C—H
N C—CH₃
N
H
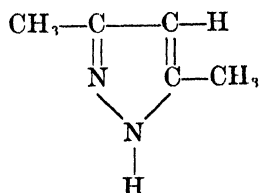
It was a relatively weak fungicide both in glass-slide and tomato-foliage tests. By substituting a nitroso (–NO) group in the 4-position, a moderately active compound (30J) was obtained. Comparable activation could not be obtained by substituting other groups such as nitro (–NO₂), amino (–NH₂) or phenylazo (–N = NC₆H₅) in this position. Compound 1E1 was activated slightly by substitution of a *p*-chlorophenyl group in the 1-position to produce J54. The full benefit of substitution in this position, however, was realized only on those pyrazoles containing a 4-nitroso group. The effective groups in the 1-position, in descending order of activation, were α-naphthyl, phenyl, methyl, and hydrogen. The effectiveness of the 1-phenyl group was further enhanced by substitution of a chlorine atom or methyl group in the *para* position to produce J49 and 2K5. The phenyl group did not exert a comparable effect when in the 5-position.

The methyl groups in the 3- and 5-positions occur as an inevitable result of the method of synthesis used. They are not considered as strong contributing forces to the fungitoxicity of the compounds. It was found, however, that the replacement of one of the methyl groups by a longer carbon chain, a *n*-hexyl group, resulted in a less effective fungicide.

The pyrazole nucleus cannot be altered appreciably without loss of fungitoxicity. When the nitroso group was substituted in the 4-position of a 3-pyrazolone as in 49K instead of in the 4-position of a pyrazole, a very weak fungicide was produced. Both the nitroso group and the pyrazole nucleus are essential for fungicidal effectiveness.

The compounds used in synthesizing these potent fungicides are relatively inactive. Isonitrosoacetylacetone was practically inert at the dosages employed in these tests, and the various hydrazines were only weakly fungitoxic.

None of the data obtained provides a suggestion as to why the 4-nitrosopyrazoles are fungitoxic. Insofar as can be determined, pyrazoles do not occur in nature (11); so there are no known analogs or comparable structures that might be blocked as reported by Woods (32) for the blocking of *p*-aminobenzoic acid by sulfanilamide. Neither is there reason to assume

that they prevent carbohydrate metabolism by converting reducing sugars into osazones, as might occur if they hydrolyzed to a hydrazine and isonitrosoacetylacetone. All of the available evidence suggests that the nitrosopyrazoles act as a unit. The nitrosopyrazoles may operate by interfering with reversible oxidation-reduction systems within a fungus cell. They, like many other good fungicides such as sulfur and quinones, are strong oxidants. More data are required, however, before drawing conclusions.

The more effective members of the 4-nitrosopyrazole series probably are the most active organic fungicides revealed to date. There are a dozen members that perform as well in laboratory and greenhouse tests as standard fungicides. Three members (J49, 2K5, 19K) are two to three times as effective in protecting tomato foliage as 2,3-dichloro-1,4-naphthoquinone, a material that controls apple scab at a dosage of 0.75 lb. per 100 gal. Their performance would classify them as superior in toxicity to other nitrogenous fungicides such as the quaternary ammonium (10, 16), glyoxalidine (29), 8-hydroxyquinoline (24), and N-nitrosophthalimidine (15) compounds. It is interesting to note that the glyoxalidines and pyrazoles have certain similarities. Both contain five-membered heterocyclic rings with two nitrogen atoms; but a carbon atom occurs between the two nitrogens in the glyoxalidine nucleus. A long-chain alkyl group is required to activate the glyoxalidines (29), and specific groups must be substituted on the pyrazoles to attain maximum fungitoxicity.

In a series of greenhouse and field trials, excellent seed protection was obtained from use of 36L, 2K5, J49, and other members of the series. The performance of these materials on seed of peas, maize, spinach, and beets was fully equal to that of standard commercial seed treatment materials. In general, the performance of 4-nitrosopyrazoles as seed protectants was in keeping with their fungicidal activity on glass slides and tomato foliage. However, one compound, 1-p-sulfamylphenyl-3,5-dimethyl-4-nitrosopyrazole (36L), which provided exceptional protection to seed, had given weak inhibition of spores on glass slides and only moderate control of leaf blight on tomatoes.

As a foliage spray, J49 gave almost perfect control of apple scab at a dosage of 4 oz. per 100 gal. It failed, however, to control cedar-apple rust and was only partially effective against *Phytophthora infestans* on potatoes and tomatoes.

The 4-nitrosopyrazoles are considered as having considerable commercial possibilities. Their greatest handicaps, from the information available, are a tendency to cause dermatitis, and a moderately high manufacturing cost.

SUMMARY

By analysis of the relations of chemical structure to fungitoxic action, it has been possible to create a remarkably effective group of new fungicides, the 4-nitrosopyrazoles. Maximum efficiency was attained by substituting an aryl group in the 1-position of 3,5-dimethyl-4-nitrosopyrazole.

The more active members of the series prevent spore germination on glass slides at concentrations as low as 0.1 ppm; provide 95 per cent protection against *Alternaria solani* on tomato foliage at concentrations of 10 to 60 ppm in spray suspension; give commercial control of apple scab at a concentration of 4 oz. per 100 gal. of spray mixture; and match, or excel, the performance of standard commercial fungicides in protecting seed of peas, maize, spinach, and beets from seed-decay and damping-off organisms in the soil.

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RELATIVE ABUNDANCE OF PHYTOPHTHORA CINNAMOMI IN THE ROOT ZONES OF HEALTHY AND LITTLE-LEAF–DISEASED SHORTLEAF PINE[1]

W. A. CAMPBELL

During the spring and summer of 1948, *Phytophthora cinnamomi* Rands was isolated from the fine roots of littleleaf-diseased shortleaf pine (*Pinus echinata* Mill.).[2] Later in the year the development of a method for assaying soil for the presence of *P. cinnamomi* made it possible to determine the relative abundance of this fungus in the root zones of both healthy and littleleaf-diseased trees.[3] By this method, which involves the use of apples for the selective isolation of *P. cinnamomi*, soil was assayed under 20 healthy trees on littleleaf-free sites, 20 healthy trees on littleleaf sites, and 20 trees with typical or advanced littleleaf. The results are given in table 1.

TABLE 1.—*Relative abundance of Phytophthora cinnamomi in the root zones of healthy and littleleaf-diseased shortleaf pine*

Tree condition	Stand condition	Total trees sampled	Soil samples per tree	Soil samples yielding *P. cinnamomi*—average per tree	
		Number	*Number*	*Number*	*Per cent*[a]
Healthy	Healthy	20	24	1.1[b]	4.6
Healthy	Littleleaf	20	24	3.5[c]	14.6
Littleleaf	Littleleaf	20	24	10.0[d]	41.7

[a] The difference between each pair of percentages is statistically highly significant by the Chi Square Test.
[b] Range 0 to 4 per tree   No Phytophthora was isolated from the soil under 10 trees.
[c] Range 0 to 8 per tree.   No Phytophthora was isolated from the soil under 5 trees.
[d] Range 4 to 18 per tree.

Soil samples were taken during December, 1948, and January and February, 1949. The weather during these months was comparatively mild, with only two periods of short duration when the minimum temperatures were much below freezing. *P. cinnamomi* was readily isolated from soil samples during the entire 3-month period. Soil samples from all types of stand and tree condition were taken concomitantly so that weather conditions that might influence isolations would be reflected in both healthy and diseased material.

Twenty-four soil samples were taken under each tree; each to a depth of 5 to 6 in. after the top inch of litter and humus had been cleared away.

These samples were taken at 2-ft. intervals starting from the base of the tree; six in the direction of each cardinal compass point.

Selections of healthy trees from healthy stands were made within a radius of 20 miles north, east, and west of Athens, Georgia. These stands ranged in age from 60 to 100 years. Selections of healthy and littleleaf-diseased trees from littleleaf stands were made from areas near Athens, Flowery Branch, Carnesville, and Elberton, Ga., and Union, Walhalla, and Abbeville, S. C. These trees ranged from 20 to 60 years of age.

The data obtained in this study demonstrate that *P. cinnamomi* is present to a limited extent in the soil around healthy trees in stands that are free of littleleaf but that it is much more abundant in the root zone of littleleaf trees. This is considered strong evidence that *P. cinnamomi* is involved in the fine root necrosis that is known to accompany the disease.[4]

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    AND
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[4] Copeland, O. L. A study of sizes, condition, and distribution of shortleaf pine roots in five Piedmont soils. Unpublished report. 1948.

FUSARIUM WILT OF STAGHORN SUMAC

E. RICHARD TOOLE[1]

Staghorn sumac (*Rhus typhina* L.) is a woody shrub that occurs widely in the Northeast, west through Ontario to eastern North Dakota, and south along the Appalachian range to Alabama. It is a source of tannin, an important game food plant, and is valued as an ornamental because of its brilliant fall coloring and its crimson fruit clusters that persist through the winter.

During the summer of 1946, patches of wilting and dead sumac were found along the Blue Ridge Parkway, near Waynesboro, Virginia. Field examination showed that numerous sumac clumps were affected in an area of about 5 acres. A *Fusarium* was consistently isolated from discolored wood of diseased material. In 1947, many more sumac plants were found affected in the original area, and scattered affected shrubs were observed up to about 3 miles north and 7 miles south from this point. A brief report of this disease was made before the 1948 annual meeting of the Southern Division of The American Phytopathological Society,[2] and a description of the causal fungus, *Fusarium oxysporum* forma *rhois* Snyder and Hepting, and its behavior in culture has been made by Snyder *et al.*[3] Scouting in 1948 disclosed a few wilted plants a few miles north of those reported in 1947, but within the wilt area no appreciable spread of the disease had taken place since 1947. In the fall of 1948, Hepting observed staghorn sumac plants with wilt symptoms in New Haven, Connecticut. Typical cultures of *F. oxysporum* f. *rhois* were isolated from the discolored vascular systems and from sporodochia fruiting on some of the diseased stems. This disease has not been reported from any other localities to date or on any other species of *Rhus*. The present paper describes the symptoms and etiology of the Fusarium wilt of staghorn sumac.

SYMPTOMS

The first external symptom of the disease is wilting of some of the leaves. The wilted leaves hang sharply downward from the twigs (Fig. 1, A) and may remain attached to the stem for some time, making it easy to detect diseased plants from a considerable distance. Death usually closely follows the wilting of the foliage. In cases where wilting is more gradual,

[2] Toole, E. R., W. C. Snyder, and G. H. Hepting. A new Fusarium wilt of sumac. (Abstr.) Pathopath. 38: 572. 1948.

[3] Snyder, W. C., E. R. Toole, and G. H. Hepting. Fusaria associated with mimosa wilt, sumac wilt, and pine pitch canker. Jour. Agr. Res. [U.S.] 78: 365–382. 1949.

FIG. 1.   A. Left, wilted sumac tree; right, a smaller healthy tree (× 1/10).   B. Vascular discoloration (see arrows) in wilted sumac: left, root piece; right, two stem pieces.

dwarfing, chlorosis, and premature autumn coloration may precede wilting.   Sporodochia of *Fusarium oxysporum* f. *rhois* sometimes occur in the

lenticels on the bark of wilted trees growing in dense stands on moist sites.

Wilting alone is not always sufficient evidence that the *Fusarium* is present, since a few plants observed with this symptom have been damaged by other causes, such as mildew and boring insects. Wilt-affected sumac has a brown discoloration in the outer sapwood, just beneath the bark (Fig. 1, B). This discoloration is greatest in the roots and lower part of the trunk, and in early stages of the disease may be sparse or lacking farther up the trunk or in the branches. It is often present only on one side in both roots and stem. Vascular discoloration has been found to extend from the roots to the petiole and base of the fruit cluster in some instances. However, there is no evidence to indicate that the fungus is seed-borne. Sections through the discolored vascular tissue show fungus hyphae in the xylem elements.

<div align="center">ETIOLOGY</div>

Fusarium oxysporum f. *rhois* was readily isolated from discolored vascular tissues. Although several morphologic strains of the fungus appeared in laboratory cultures, only one morphologic strain has been isolated from nature, and this one was used throughout the pathogenicity tests.

The pathogenicity of *Fusarium oxysporum* f. *rhois* was tested by a series of experiments. In experiment 1, seed from a wilting staghorn sumac collected in August 1947 was treated with sulfuric acid and planted in the greenhouse in flats containing a mixture of sand and vermiculite. When the seedlings were 1 month old, 13 of them in one flat were inoculated by pouring a few milliliters of a spore suspension of *F. oxysporum* f. *rhois* into a small hole made in the soil near the base of each plant. Another flat containing 20 seedlings from this same seed source served as a control. First symptoms of wilt appeared in the inoculated seedlings after 3 weeks, and all inoculated seedlings were dead within 2 months, while controls remained healthy (Table 1). The *Fusarium* used as inoculum in the soil was

TABLE 1.—*Susceptibility of different species of Rhus to Fusarium oxysporum f. rhois*

Experi- ment	Age of stock	Tree species	Inoculated		Control	
			Total trees	Trees wilted[a]	Total trees	Trees wilted
			Number	*Number*	*Number*	*Number*
1	1 month	*Rhus typhina*	13	13	20	0
2	1 year	Do	30	22	10	0
3	2 years	Do	3	3	..	
4	4 months	*R. typhina*	18[b]	18	6	0
	4 months	*R. glabra*	18	0	6	0
	4 months	*R. copallina*	18	0	6	0
5	6 months	*R. typhina*	18	10	6	0
	6 months	*R. glabra*	18	0	6	0
	6 months	*R. copallina*	18	0	6	0
6	1 year	*R. toxicodendron*	15	0	5	0

[a] Original fungus reisolated from wilted trees.
[b] All 18 trees showed vascular discoloration at end of experiment.

reisolated from the vascular tissue of all of the wilted seedlings, and micro-
scopic examination showed mycelium present in the vascular elements of the
wilted plants.

Seed from healthy *Rhus typhina* in the wilt area of Virginia were used
in experiment 2. Ten seedlings were planted in September 1947 in each
of four metal flats in a sand and loam mixture, and allowed to grow in the
greenhouse. In April 1948, the seedlings in three of the flats were inocu-
lated as in experiment 1. Those in the fourth flat served as controls. The
results to date appear in table 1. First wilt symptoms were observed in
less than 1 month after inoculation. After 2 months, 7 of the 20 trees in
two of the three inoculated flats had wilted and other trees were somewhat
dwarfed and chlorotic (Fig. 2).

FIG. 2. No inoculum was added to soil in the flat on the right. Two months before
the photograph was taken, inoculum was added to soil in the two flats on the left; 7
of the 20 trees in these two flats had wilted at time of photographing.

In August 1947, three 2-year-old plants of *Rhus typhina* growing in
North Carolina several hundred miles south of the Virginia wilt area were
transplanted to a sand bench in the greenhouse. These trees were allowed
to grow undisturbed until April 1948, at which time they were used in ex-
periment 3. All 3 trees were inoculated by pouring a few milliliters of a
spore suspension of *Fusarium oxysporum* f. *rhois* over the sand near the
base of the trees. All had wilted in 3 months (Table 1).

Experiment 4 was undertaken to compare the susceptibility of *Rhus
glabra* L. and *R. copallina* L. with *R. typhina*. On April 15, 1948, six 4-
month-old seedlings of each of the three species were transplanted to each
of four flats containing a mixture of sand and vermiculite. The seedlings
in three of the flats were inoculated with *Fusarium oxysporum* f. *rhois*
by dipping the root systems in a spore suspension of the fungus at the
time of transplanting. Those in the remaining flat were dipped in sterile
water before planting, and served as controls. Two and a half months
later, all seedlings were dug up and the root systems examined. Only
seedlings of *R. typhina* showed wilt and vascular discoloration (Table 1).

Experiment 5 was a repetition of experiment 4, with additional seed-

lings from the same seedling stock after it had grown 2 months longer. The results agree closely with those of the previous experiment (Table 1).

In experiment 6, the susceptibility of *Rhus toxicodendron* L. to the sumac wilt *Fusarium* was tested. Five 1-year-old seedlings were planted in each of four metal flats in a sand and loam mixture on July 31, 1947. The seedlings in three of the flats were inoculated at the time of planting by dipping the roots in a spore suspension of *Fusarium oxysporum* f. *rhois,* while those planted in the fourth flat were not inoculated. One year later, additional inoculum was added to the soil in the inoculated flats. No wilt has appeared after two seasons (Table 1).

The sumac wilt fungus, *Fusarium oxysporum* f. *rhois,* and the mimosa (*Albizzia*) wilt fungus, *F. oxysporum* f. *perniciosum* (Hepting) Toole,[3] appeared identical in culture, and therefore it was considered necessary to cross-inoculate with these two fungi on the two host species to determine if either fungus could infect both hosts. This was done in the fall of 1947 and repeated in 1948. Sumac plants were wilted only by the sumac *Fusarium,* and Albizzia plants were wilted only by the Albizzia *Fusarium.*

DISCUSSION

Inoculation experiments indicate that for the four species of *Rhus* tested, *Fusarium oxysporum* f. *rhois* is specific and will infect only *Rhus typhina,* while *R. glabra, R. copallina,* and *R. toxicodendron* remain unaffected by it. Likewise, this fungus will not infect *Albizzia julibrissin* Durazz., nor will the Albizzia (mimosa) wilt fungus infect staghorn sumac, although the two fungi can not be distinguished from each other in culture. Age of stock apparently had little effect on the development of the disease in these experiments; however, further experimentation is needed to determine the full effect of age of stock as well as soil texture, temperature, moisture, and other factors on disease development.

There appears to be a difference in wilt-susceptibility among different plants of *Rhus typhina.* All of the plants inoculated in experiments 1 and 3 wilted in 3 months, while 73 per cent had wilted after 6 months in experiment 2, 72 per cent in $2\frac{1}{2}$ months in experiment 4, and 55 per cent after 4 months in experiment 5. Because of this apparent variability in resistance, the selection and propagation of wilt-resistant clones of *R. typhina* may be possible. Root cuttings of this species root readily.

The fact that the check plants grown from seed from a wilting sumac remained healthy indicates that this disease may not be seed-borne. The limited geographic distribution of the disease makes it difficult to evaluate its importance at this time. However, it is interesting to note that this is only the second *Fusarium* vascular wilt of a woody plant species yet reported.

SUMMARY

A vascular wilt of staghorn sumac (*Rhus typhina*) caused by *Fusarium*

oxysporum f. *rhois* has been found in Virginia and Connecticut. It is characterized by wilting of the foliage, soon followed by death of the affected plant. Inoculation experiments indicate that this fungus will wilt *R. typhina,* but not *R. copallina, R. glabra, R. toxicodendron,* and *Albizzia julibrissin,* and that possible strain differences exist in *R. typhina.*

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PHYTOPATHOLOGICAL NOTES

Zonate Leaf Spot of Sorghum in Venezuela.[1]—In September 1947 in plots of grain sorghum (*Sorghum vulgare* Pers.) in Maracay, Aragua, Venezuela, a new kind of leaf spot was found. Dr. C. O. Grassl of the United States Department of Agriculture had sent the seed to Venezuela for experimental purposes, with the advice that it be treated with a dust fungicide before planting; this, however, was not done. The sorghums under test had been collected in Asia and Africa and multiplied in Florida or at Beltsville in the United States. Later this disease became quite common and could be found in other places also. The lesions were subcircular spots with alternating dark and light bands and, in the first hours of the morning, a watersoaked halo. In a moist chamber and within 24 hr., yellow-gray clusters of conidiophores in longitudinal lines appeared in the light zones. These cespitula were minute, pinkish columns, formed by a number of filiform, pluriseptate, arcuate, non-branched, hyaline conidia, which were upright and parallelly juxtaposed on short conidiophores. These characters suggested that the fungus was *Gloeocercospora sorghi* Bain and Edg., described by these authors[2] as the cause of the zonate leaf spot of sorghum and other grasses in the United States. Dr. Lindsay S. Olive kindly confirmed the diagnosis.

The measurements of conidia were taken in lactophenol, after the leaves had been held for 10 days in a moist chamber at room temperatures (14°-27° C.). The average length of the conidia was 102.5 μ with 139.0 μ maximum and 46.5 μ minimum. The width was from 1.4 μ to 3.0 μ. In other observations conidia of 148.0 μ were seen. Some differences were observed in the length of the conidia, that showed a narrower range than in the type material, and in the unreliability of the slimy matrix about the conidia as a distinguishing feature. The latter character appeared late in the moist chamber and was not seen in the field, possibly because of environmental conditions in Venezuela. In potato-dextrose-peptone agar, slimy conidia were easily obtained. In the field sclerotia were not seen, probably because the leaves decay quickly in these tropical regions and the parasite has not sufficient time to differentiate the resting stage. Sclerotia (80–190 μ), however, formed in naturally infected leaves when these were kept in the moist chamber for a sufficient length of time; and on potato-dextrose-peptone agar, sclerotia appeared within 10–12 days.

Differences in susceptibility to the fungus were noted among the different strains and populations of sorghum grown in the same experimental plot. With two varieties, Milo and Shrock, periodical plantings were

[2] Bain, D. C., and C. W. Edgerton. The zonate leaf spot, a new disease of sorghum. Phytopath. **33**: 220–226. 1943.

made throughout the year. The infection was especially severe during September, October, and November, gradually became milder in later plantings, and in the driest months from January through March the disease disappeared completely. It was seen again with the new rainy season in June.

This is the first report of *Gloeocercospora sorghi* in South America. Outside of the United States there is a previous record in Tanganyika, Africa, which seems to be based on sclerotia only.[3, 4]—ANTONIO CICCARONE, Division de Fitopatología, Ministerio de Agricultura y Cría, Maracay, Venezuela.

Sporulation of Gibberella zeae on Rice Straw.[1]—Head scab of wheat, caused by *Gibberella zeae*, is a common disease in humid regions in China and may cause considerable damage.[2] One of the most commonly grown varieties of wheat, No. 2905, which yields well and possesses other good agronomic characters, is very susceptible to scab.

The most practical measure for control of head blight is the growing of resistant varieties, but in testing varieties for resistance to scab, an abundance of spores is essential for good infection. Dickson[3] reported that *G. zeae* produced macroconidia readily on potato-dextrose agar, and Wollenweber[4] stated that conidial or perithecial stages of this fungus were found on various substrates. Apparently the Chinese isolates of *G. zeae* are different from the European and American isolates, because Chinese workers have failed to secure sporulation in various media. In this study conditions conducive to production of conidia were ascertained.

The isolate of *G. zeae* used was collected in Chengtu, Szechwan Province, in April, 1939. Sporulation on eight natural substrates was studied: cabbage, potato, tomato stem, eggplant stem, wheat kernel, cotton stem, and sweet potato cylinder. Fresh material was sterilized directly, but whenever dry plant parts were used they were soaked in tap water for two hours before sterilization. A definite amount of material taken from each of five flasks, incubated at 24° C. for 12 days, was examined for macroconidia. At this temperature the fungus formed white and pinkish mycelium with numerous microconidia on all substrates except wheat kernels. No macroconidia were observed in any of these cultures.

The cultures were kept for a longer time at 24° C. and examined at

[3] Wallace, G. B., and Maud M. Wallace. Tanganyika Territory fungus list: recent records. VI. Mycol. Circ. Dept. Agr. Tanganyika 15. 1945.

[4] Wallace, G. B. Report on a plant disease survey in the Lake and Western provinces and on the Central Railway. March–April 1945, by the plant pathologist. Mycol. Circ. Dept. Agr. Tanganyika 7. 1945.

[1] This work was done at the University of Nanking.
The writer is indebted to Professors C. T. Wei and C. K. Lin for their directions during the progress of the work.

[2] Wu, Y. S. [A report of a survey of wheat diseases.] In Chinese. Nung Pao 3: 1835–1889. 1936.

[3] Dickson, J. G. Production of conidia by *Gibberella saubinetii.* Jour. Agr. Res. [U.S.] 19: 235–237. 1920.

[4] Wollenweber, H. W. Studies on the Fusarium problem. Phytopath. 3: 24–50. 1913.

intervals of five days. On December 26, 1940, 45 days after inoculation, many macroconidia were found on the rather dry sweet potato cylinder. Cultures on other media failed to produce macroconidia within two more months.

In other tests substrates made from corn kernels, corn cob, barley kernels, barley chaff, wheat straw, wheat rachis, and rice straw were inoculated. After incubation at 24°–26° C. for 4 days, macroconidia were present on the rice straw culture. Results were the same in repeated tests.

Cultures on rice straw were incubated at room temperature (approximately 12° C.), 16°, 20°, 24°, 28°, and 32° C. for 20 days. They were examined for sporulation at intervals of two days. Between 16° and 28° C. cultures sporulated rapidly and macroconidia were abundant; at 12° C. sporulation was delayed and few macroconidia were formed; and at 32° C. cultures failed to grow.

In the spring of 1941 cultures grown on rice straw in flasks at approximately 28° C. yielded few macroconidia and it was believed that the medium became too dry because of excessive evaporation. To overcome this, flasks were prepared, containing 25 cc. of 1 per cent agar; after sterilization, in each flask 1 gm. of dry rice straw with 1 cc. of tap water, both previously sterilized, were placed on top of the solidified agar, then two drops of spore suspension containing 10 to 15 macroconidia of *G. zeae* were added as inoculum. The cultures were incubated at 28° C. for 27 days. Three flasks were sampled and macroconidia were counted every third day. Each flask was shaken thoroughly after receiving 20 cc. sterile distilled water, and 5 cc. of the spore suspension were withdrawn for microscopic examination. Macroconidia were counted in five fields under the low-power objective and means were determined for the 15 counts from triplicate samples. Spores were most numerous between the sixth and fifteenth days, but counts declined thereafter.

To secure quantities of macroconidia of Chinese isolates of this fungus, the writer suggests that it be grown for 9 to 18 days on rice straw at 28° C. with sufficient moisture —S. T. CHEN, Department of Agronomy, College of Agriculture, National University of Shantung, Tsingtao, China.

SYNONYMY IN SOME COMMON SPECIES OF CERCOSPORA[1]

E. M. JOHNSON AND W. D. VALLEAU

Frogeye of tobacco, usually attributed to the fungus *Cercospora nicotianae* Ell. and Ev., is a common but seldom destructive disease, usually on lower leaves of plants in humid locations. In a damp autumn, infections may occur on higher leaves and, if the crop is cut before it is fully ripe, green spots (2) may appear on the cured leaves; but if the crop is allowed to ripen, the upper leaves may suddenly develop large necrotic spots and be quickly destroyed unless cut immediately. Frogeye tobacco is usually not discounted on the market, because buyers take the view that the spots are an indication of a ripe crop.

Careful observations at setting time over a period of 25 years have shown that frogeye is an extremely rare disease in plant beds in Kentucky. We occasionally have found a single frogeye spot at the time of first pulling, but usually all plants in the beds appear to be completely free from infection. Yet in the field the disease is comparatively common late in the season.

These observations raise the question whether the causal agent, *Cercospora nicotianae,* is strictly a pathogen of tobacco or whether it might not also be a pathogen of other plants from which wind-borne spores might be carried to tobacco. Whether *C. nicotianae* is a valid species, restricted in its host range, is of fundamental importance to the pathologist who is trying to determine the source of infection. The same, of course, is true of the fungi causing leaf spots of beet, celery, and other commercial crops that are regularly injured by cercospora leaf spot. If *C. nicotianae* attacks only tobacco and closely allied plants, then control measures can be based largely upon a study of the organism in relation to tobacco. If, however, *Cercospora* from numerous host species attacks tobacco, as Vestal (8) has shown for sugar beets, then the disease on tobacco must be studied from the standpoint not only of tobacco but of the native vegetation as well.

The purpose of this report is to show that the inoculum for outbreaks of frogeye in tobacco need not arise from a previous tobacco crop, but may originate on several unrelated plants growing in the vicinity of tobacco plant beds and fields; and that, as a consequence, the usual concept of species in *Cercospora* must be revised.

MATERIALS AND METHODS

Cultures of *Cercospora* were obtained by streaking on potato-dextrose agar conidia from cercospora leaf spots from 28 species of plants in 16

763

TABLE 1.—*Results of inoculations with Cercospora isolated from crop plants and weeds*

Source of isolate	Number of plants infected: number inoculated[a]							
	Tobacco	Bean	Cabbage	Beet	Petunia	Nicotiana rustica	Pansy	Cantaloupe
Solanaceae								
Nicotiana tabacum L.	17/19	6/11	11/15	15/23	3/11	43/52	0/1	0/1
Petunia hybrida Vilm.	1/3	3/4	2/3	4/6	0 4	7/12		1/1
Physalis subglabrata Mack. and Bush.	2/3	2/3	1/1	1/3	1/2	0/3		
Campanulaceae								
Campanula sp.	1/1	0/5	1/2			1/1	1/1	0/1
Chenopodiaceae								
Beta vulgaris L.	0/1	5/6	3/3	4/4	1/3	1/3		0/1
Chenopodium album L.		3/3	0/1	1/1	0/2			
Compositae								
Chrysanthemum parthenium Pers.		1/1	0/1	1/1	0 1			
Coreopsis sp.	1/1	0/3	0/1		0/1	0/2	1/1	0/1
Convolvulaceae								
Ipomoea hederacea Jacq.	1/1	1/1	2/2			0/1		0/1
Cruciferae								
Brassica oleracea var. capitata L.	2/2	2/2	2/4	1/1	1/2	1/1		
Barbarea vulgaris R. Br.	1/1	3/3	0/2					
Cucurbitaceae								
Cucurbita pepo L.	1/3	1/2	2/2	4/4	2/3	10/11		0/1
Cucumis sativus L.		1/4	1/2			1/1		1/1
Euphorbiaceae								
Ricinus communis L.		1/1	0/1		0/1			0/1
Gramineae								
Festuca elatior var. arundinacea (Schreb.) Wimm.		1/1	1/1	1/1	0/1			0/1

TABLE 1—(Continued)

Source of isolate	Number of plants infected: number inoculated[a]							
	Tobacco	Bean	Cabbage	Beet	Petunia	Nicotiana rustica	Pansy	Cantaloupe
Labiatae								
Lamium amplexicaule L.	1,1	3/3	2/2	2/2	1/1	4/4	2/2	0/1
Leguminosae								
Lathyrus latifolius L.	1/1	2/3	2/3	2/2	1/1	0/2	1/1	1/1
Lupinus polyphyllus Windl.	1/2	4/4	2/3	6/7	3/4	11/16	1/1	1/1
Phaseolus vulgaris L.	1/2	4/6	3/4		1/2	0/1	1/1	1/1
Trifolium hybridum L.	0/1	0/4	0/2	1/1	0/1			0/1
Trifolium repens L.		1/2						
Malvaceae								
Malva rotundifolia L.	0/1	1/3	1/2	0/1	0/2	1/3		0/1
Althaea rosea Cav.	0/1	0/1				0/1		
Phytolaccaceae								
Phytolacca decandra L.	2/3	1/5	2/2	1/4	0/3	2/8		1/1
Plantaginaceae								
Plantago lanceolata L.	0/2	2/5	0/1	1/1	0 1	4/8		0/1
Polemoniaceae								
Phlox paniculata L.	1/2		2/2			1/1		
Rosaceae								
Potentilla sp.	0/1		0/2	1/1	1/1	0/2		1/1
Rosa sp., var. Blaxe		0/1	2/2	0/1		1/1	2/2	
Rubus allieghensis Porter	0/1	1/1	0/2			1/2		0/1
Violaceae								
Viola sp.	1/3	3/4	5/6	3/3	1/2	6/7		

[a] Numerator—number of plants infected; denominator—number of plants inoculated.

families (Table 1). The isolates were grown on tobacco agar (3) at 27°
C. in the dark. Inoculations were made to tobacco, *Nicotiana rustica*, and
to other plants (Table 1). Mycelial cultures were used as inoculum, as
spores were sparse or absent. Actively growing potted plants were inocu-
lated by dipping their leaves in a mixture of 10 cc. of liquid soap in 400
cc. of water as a wetting agent, then in a suspension of a 3- to 5-day-old
tobacco agar culture prepared by squeezing the agar mat through cheese-
cloth. After 48 hr. in a moist chamber the plants were kept in the green-
house until spots developed, when they were returned to the moist chamber
for 24 hr. Cercospora conidia produced on the spots were considered
proof of pathogenicity. Camera lucida drawings were made of spores from
culture and from inoculated plants for ready comparison of the so-called
species.

<div align="center">RESULTS</div>

In culture, colonies of the isolates from the various hosts were so similar
that they could not be distinguished. Variations in color and in the
amount of aerial mycelium between the so-called species were matched by
variation within the isolates from a single host. Camera lucida drawings
of spores and sporophores from the various hosts on which the isolates
were grown gave no basis for separation of any of the isolates. From the
standpoint of colony characters and morphology of conidia and conidio-
phores produced in culture, all of the isolates appear to be of a single
species.

Frogeye spots that developed on Burley tobacco from inoculation with
isolates from 16 host species in 11 families (Table 1) appeared alike and
were typical of the disease as it occurs in the field. Measurements of
spores and conidiophores from the lesions varied as much among the iso-
lates from a species as among isolates from different species. Some dif-
ferences were indicated in degree of pathogenicity among the isolates tested,
but these differences were no greater than those among the 6 isolates from
tobacco.

Scotia bean became infected when inoculated with isolates from 23
plant species in 15 families. The spots were alike and there were no con-
sistent differences in spore measurements.

Isolates from 19 plant species in 14 families produced infection on Wis-
consin All Seasons cabbage. The spots were grayish, depressed, irregular
to circular, and varied from 4 to 10 mm. in diameter. Often an entire
leaf was killed. Neither conidia nor conidiophores from the different iso-
lates could be distinguished.

Seventeen of 19 isolates from 12 families caused typical cercospora leaf
spot on Detroit Red beet. The spots from different isolates could not be
distinguished, and again spores and conidiophores could not be distin-
guished.

Isolates from 11 species representing 8 families caused infection on

Heavenly Blue petunia: Two isolates from petunia failed to cause infection on petunia but they infected tobacco, bean, cabbage, and *Nicotiana rustica.*

Seventeen isolates from different host species representing 15 families caused typical frogeye infection on *Nicotiana rustica.* The spots were somewhat smaller than those occurring naturally in the field. Again the isolates could not be distinguished on the basis of conidiophores or conidia.

Only 7 isolates from different plants in 5 families were tested on Alpine Glow pansy, but all 7 caused spots typical of those that occur on *Viola sp.* in nature. Isolates from 6 plant species of 19 tested caused infection on cantaloupe.

It is evident from these inoculation tests that several so-called species of *Cercospora* from unrelated hosts cause typical frogeye on tobacco.

SYNONYMY IN CERCOSPORA

Mycologists seem to have assumed a high degree of specificity of *Cercospora* and a relatively high degree of stability of the organism in size of spores and conidiophores. When *Cercospora* is found on a plant on which it has not previously been reported, this is taken as sufficient basis for announcing a new species. If *Cercospora* has already been described on this host, but the collection does not correspond in morphological characters with the one described, this seems to justify the description of a new species, regardless of the fact that there may be morphologically similar species on members of many other plant families or even on other genera and species within the family.

While parasitism seems to be considered of first importance in mycological literature, *Cercospora* is in fact a weak parasite living, as Atkinson (1) pointed out, on dead, dying, languid, physiologically diseased tissues with perhaps occasional injury to healthy plants. Recognizing that different hosts, because of structural differences, might exert a powerful influence upon the form and characteristics of *Cercospora,* and considering that these might be effective agencies in producing variations in the parasite, Atkinson conceived that during a long period of time a few forms widely distributed over a great number of hosts may become more and more unlike and more firmly fixed in the possession of peculiar characteristics. He was assuming, without evidence, that the various forms remain generation after generation on the same host; whereas the results of cross-inoculation studies make it almost certain that any one of the forms of this semiparasitic organism are continually moving about from one host species to another. Thus changes in morphology are merely temporary ones forced upon the fungus by the temporary host. A fungus living under these conditions could hardly possess a high degree of host specificity.

That neither host nor minor differences in morphology are sound bases

for classifying *Cercospora* is suggested by the work of Welles (9). His inoculation of 13 species representing 12 genera of plants in 5 families with *Cercospora* from *Phaseolus lunatus* resulted in infection on all of the species. Welles found that spore size on the different genera varied enormously and concluded that morphological differences, unless exceedingly pronounced, are of little value as taxonomic criteria. He further concluded that the only valuable taxonomic criteria which have presented themselves for use in separating various species of *Cercospora* are physiological behavior on artificial media and extent of parasitism.

Vestal (8) found that 26 species of plants distributed throughout 12 widely separated families could be infected in the greenhouse by spraying with suspensions of conidia of *Cercospora beticola*, clearly indicating a wide host range of the organisms among plants of the sugar beet area. In the field, Vestal found six species of plants in six widely separated families infected with *Cercospora* morphologically and pathogenically similar to *C. beticola*. There is a possibility that 24 so-called species of *Cercospora* may be involved in the beet disease alone. Hill's (4) extended study of cercospora leaf spot of tobacco in Australia, in both greenhouse experiments and observations out-of-doors, convinced him that "only under exceptional circumstances do members of the Solanaceae other than the Nicotianae act as alternative hosts for the species of *Cercospora* affecting tobacco. In the majority of seasons they are not important."

The experimental results reported in this paper and the results of Welles (9, 10), Vestal (8), and Horsfall (5) indicate that some species of *Cercospora* have an extremely wide host range and that many so-called species that have been considered distinct are in fact one and the same. According to Lieneman (6), the type species of the genus *Cercospora* is *C. apii* Fres. Vestal (8) showed that *Apium graveolens* readily developed typical cercospora leaf spot when inoculated with *C. beticola*. That the two fungi are identical morphologically is well illustrated by Schwarze (7) in his drawings of conidia from the two species. It should be mentioned that while Schwarze described the spores of *C. apii* as clavate, on the basis of notes of W. S. Krout, and those of *C. beticola* as obclavate, the term clavate is obviously an error since Saccardo and others describe *C. apii* spores as obclavate. In our work we have been able to find no real morphologic differences between *C. beticola* and *C. nicotianae;* and in the inoculation studies of Vestal and in the present study both species caused infection on nine families in common, indicating that both have a very wide and similar host range. There is, therefore, ample evidence for considering *C. apii, C. beticola,* and *C. nicotianae* identical. In the limited cross-inoculation work that has been carried on, a relatively small number of so-called species of *Cercospora* has been studied. Yet the studies have been sufficient to show that the forms worked with are omnivorous and undoubtedly attack many more species of plants than we have had an oppor-

tunity to study. We propose, therefore, that all species of *Cercospora* having the general morphologic characteristics of *C. apii* be cast in synonymy with this species until proof is forthcoming that they are different. This probably will include several hundred names.

The species listed below are morphologically similar to *Cercospora apii* and on their respective hosts either *C. beticola* or *C. nicotianae* cause typical cercospora spots. They are considered synonymous with *C. apii*: *C. acetosellae* Ell., *C. alabamensis* Atk., *C. althaeina* Sacc., *C. anthelmintica* Atk., *C. arcti-ambrosiae* Halst., *C. avicularis* Wint., *C. bliti* Tharp, *C. bloxami* Berk. and Br., *C. brachiata* Ell. and Ev., *C. canescens* Ell. and Mart., *C. chrysanthemi* Heald and Wolf, *C. citrullina* Cooke, *C. columnaris* Ell. and Ev., *C. convolvuli* Tracy and Earle, *C. cruenta* Sacc., *C. cucurbitae* Ell. and Ev., *C. diffusa* Ell. and Ev., *C. festucae* Hardison, *C. filispora* Peck, *C. flagellaris* Ell. and Mart., *C. ipomoeae* Wint., *C. kellermani* Bubák, *C. lathyri* Dearn and House, *C. lathyrina* Ell. and Ev., *C. longispora* Peck, *C. lupini* Cooke, *C. lupinicola* Lieneman, *C. omphakodes* Ell. and Holw., *C. phaseolorum* Cooke, *C. phyllitidis* Hume, *C. physalicola* Ell. and Barth., *C. physalidis* Ell., *C. plantaginella* Tehon, *C. plantaginis* Sacc., *C. polygonacea* Ell. and Ev., *C. ricinella* Sacc. and Berl., *C. rosigena* Tharp, *C. rubi* Sacc., *C. septorioides* Ell. and Ev., *C. solanicola* Atk., *C. violae* Sacc., *C. viridula* Ell. and Ev., *C. zebrina* Pass.

SUMMARY

Frogeye of tobacco, caused by a species of *Cercospora*, though a rare disease in plant beds in Kentucky at pulling time, is a common and sometimes severe disease in the field. This raised the question as to whether inoculum in field outbreaks came from a previous tobacco crop or from other plants on which the fungus might also be parasitic.

Cultures of *Cercospora* were obtained from 28 species in 16 plant families. The isolates from the various hosts could not be distinguished by colony characters or by spore size or shape. Typical frogeye developed on tobacco inoculated with isolates from 16 species in 11 families. Similar results were obtained on other species of plants.

Neither pathogenicity based on host range nor morphology has been given sufficient consideration in naming new species of *Cercospora*. Species are usually named for the plant on which they are found. The evidence obtained in this study indicates that the species of *Cercospora* that attacks tobacco is omnivorous, occurring on many other host species. As *C. apii* Fres. appears to be the type species of the genus, and as *C. nicotianae* and *C. beticola* appear to be identical morphologically and pathogenically with it, it is proposed that these species and other species morphologically similar to *C. apii* be cast in synonymy with it until evidence to the contrary is forthcoming.

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EVALUATION OF RESISTANCE AND SUSCEPTIBILITY IN GARDEN PEA TO NEAR-WILT IN THE GREENHOUSE[1]

D. G. WELLS, W. W. HARE,[2] AND J. C. WALKER[3]

The common garden pea (*Pisum sativum* L.) is subject to two vascular fusarial diseases, wilt and near-wilt, incited by *Fusarium oxysporum* f. *pisi* (Linford) race 1 Snyder and Hansen, and *F. oxysporum* f. *pisi* (Linford) race 2 Snyder and Hansen, respectively. Wilt was once a serious disease but adequate resistance was reported by Wade (5) in 1929 and was later incorporated into commercially important field and canning pea stocks. According to Walker and Hare (6), near-wilt, described in detail by Snyder and Walker (3) in 1935, became a disease of major importance in Wisconsin by 1942. Finding of resistance to near-wilt was reported by Hare *et al.* (1) in 1949 and is being currently used in the production of new varieties at the Wisconsin Agricultural Experiment Station.

During the early period of breeding for resistance to near-wilt, tests for disease reaction were conducted on soil naturally infested with the organism. This system was unsatisfactory because environmental conditions were often unfavorable for the complete elimination of near-wilt susceptible plants (1). A more dependable test for reaction to near-wilt was needed in order that the breeding program might be properly conducted.

Virgin and Walker (4) demonstrated in 1939 that Wisconsin Perfection peas grown in the greenhouse in soil either naturally or artificially infested with the near-wilt organism developed severe symptoms of near-wilt in 52 days from time of planting. Soil temperatures ranging from 20° to 32° C. were maintained. By the time adequate notes on disease development could be secured, the plants had grown so tall as to be difficult to handle and greenhouse space was not being efficiently utilized. This method of testing in the greenhouse for reaction to near-wilt was thus unsatisfactory for use in a breeding program.

The purpose of this paper is to report experiments made to establish a rapid test for reaction to near-wilt under controlled conditions in the greenhouse.

METHODS AND MATERIALS

Two varieties were used in these experiments, Delwiche Commando, a recent release of the Wisconsin Agricultural Experiment Station, resistant to wilt and near-wilt, and Wisconsin Perfection, a standard wilt resistant

canning variety, susceptible to near-wilt. These varieties have been shown by Hare *et al.* (1) to differ by a single factor for reaction to near-wilt.

Inoculum was obtained by growing the fungus in 250-ml. Erlenmeyer flasks containing 100 ml. of modified Czapek's solution.[4] Flasks containing inoculum were placed on a mechanical shaker for two days and then the contents of one flask, observed to be free of contaminants, were used to inoculate 3 liters of sterile Czapek's solution contained in a 6-liter flask. Two or three such quantities of nutrient solution were inoculated in this manner and placed on a mechanical shaker for 3 days. After this period of agitation the contents of the flasks consisted of a heavy suspension of spores and mycelial fragments.

Plants were grown in two types of containers. Metal pans, $3 \times 12 \times 18$ in., were filled with clean white quartz sand to within about $\frac{1}{2}$ in. of the top. Glass siphons of the type described by Schroeder and Walker (2), installed at one end of each pan, were used in the first experiments performed but were found to be unnecessary if care was taken not to apply an excess of liquid. The pans were inserted into Wisconsin soil-temperature-control tanks.

The sand was flooded with distilled water in preparation for planting and the excess was siphoned off. A planting board was used that punched 12 holes per row, one row at a time, just deep enough for the seed to be easily covered, a distance of about $\frac{1}{4}$ in. Eight rows were planted in each pan. Shallow planting was found to be desirable for uniform emergence even though some of the seeds were forced out of the sand by the growth of the primary root before it was anchored by root hairs. Such seeds had to be placed back in the sand. Whenever the pans needed moisture they were sprinkled with nutrient solution.

Seven to 10 days from time of planting the seedlings were removed from the pans and treated as follows. Seedlings from one row (12 seedlings) of each of the varieties were immersed in distilled water, the tap roots clipped to a uniform length of 1 in., and the seedlings replanted in the pan. The roots of seedlings from two other rows were immersed in a heavy suspension of spores and mycelial fragments of the near-wilt fungus and then returned to the pan. Seedlings from the remaining rows were similarly treated except that the tap roots were clipped to a length of 1 in. while immersed in the inoculum and were then transplanted in the sand. Thus two of the eight rows were for control purposes, two were inoculated by dipping, and four were inoculated by clipping.

The clipping method of inoculation was later improved. The roots were washed in distilled or tap water and the seedlings laid a row at a time on a cutting board. This board had a groove running lengthwise deep enough to accommodate the cotyledons and to make the seedlings lie at a uniform distance from another, but narrower, groove over which the roots extended. The board was then laid on the surface of inoculum in a shallow baking

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[4] KNO$_3$, 3.0 gm.; KH$_2$PO$_4$, 1.0 gm.; Mg SO$_4$.7H$_2$O, 0.5 gm.; KCl, 0.5 gm.; FeSO$_4$, trace; cerelose, 30 gm.; water, 1,000 ml.

pan and a slender stick was placed over the seedlings to prevent their float-
ing when the board was immersed.   After immersion, the tap roots were
severed by inserting one end of a razor blade in the narrow groove and
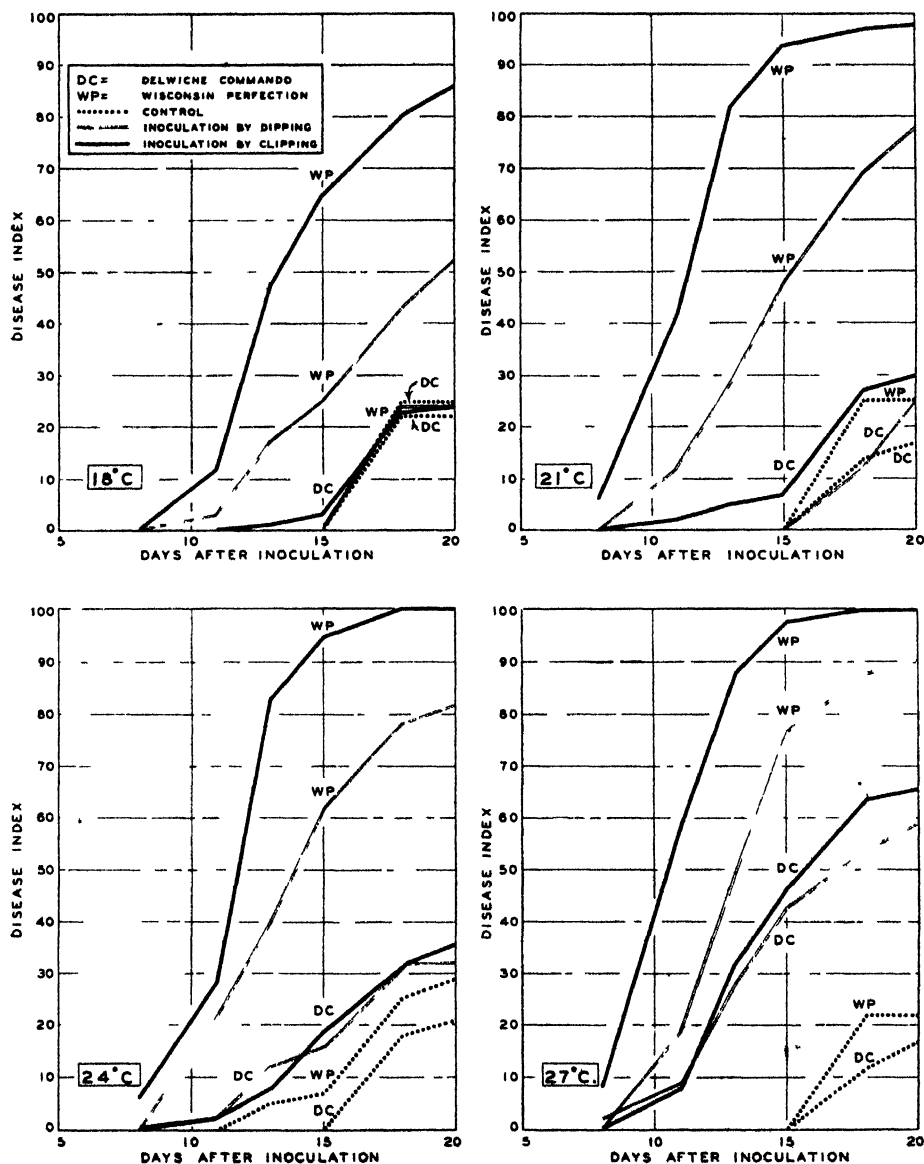
FIG. 1.   Disease index curves at indicated sand temperatures for Delwiche Commando
and Wisconsin Perfection pea seedlings inoculated 8 days after planting with *Fusarium
oxysporum* f. *pisi* race 2.

pulling it toward the operator so as to cut all of the tap roots about ⅞ in.
from the cotyledons.   A row of 12 holes 1½ in. deep were then punched in
the sand and the inoculated seedlings were quickly and easily transplanted.
When the transplanting was completed the pan was returned to the tem-
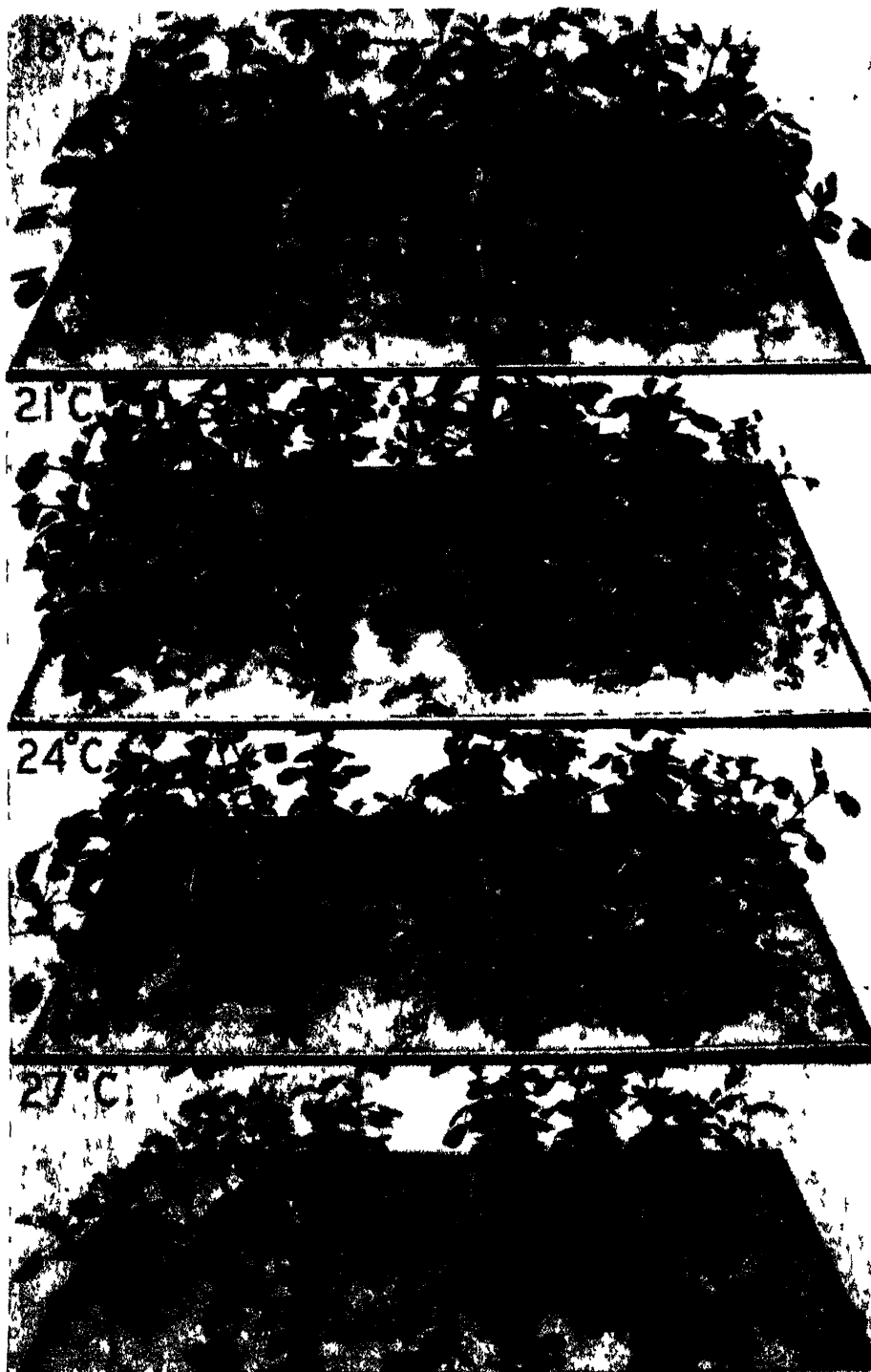
FIG. 2. Comparative development of near-wilt of peas at the indicated temperatures 20 days after inoculation with *Fusarium oxysporum* f. *pisi* race 2. Rows (numbered from left to right) 1, 3, 5 and 7, Delwiche Commando; rows 2, 4, 6, and 8, Wisconsin Perfection. Rows 1 and 2 inoculated by the dip method; rows 3 and 4, 7 and 8, by the clip method. Rows 5 and 6 were not inoculated.

perature tank and lightly sprinkled with nutrient solution, care being taken not to overwater. If the sand was wet enough so that water could be siphoned off, then a siphon was set in place and as much water was removed as possible.

Tank temperatures were adjusted to maintain sand temperatures of 18°, 21°, 24°, and 27° C., while a common air temperature of about 22° C. was maintained. The seedlings were examined at 8, 11, 13, 15, 18, and 20 days after inoculation and each was assigned to one of four disease classes: Class 1, healthy—normal color and development; Class 2, slightly diseased—one stipule of a pair smaller than the other, or yellowing and dying of one-half or fewer of the leaves at the plant base and upward; Class 3, moderately diseased—more than one-half of leaves dead and remainder yellowing and wilting, often unilaterally, except at the green growing tip; Class 4, dead—dead plant tip.

Disease indices were calculated for each row of each treatment by the method used by Walker and Foster (7).

EXPERIMENTAL RESULTS

Disease development curves for Delwiche Commando and Wisconsin Perfection in control rows and in rows inoculated 8 days after planting by the dip and clip methods at sand temperatures of 18°, 21°, 24°, and 27° C. are shown in figure 1. These data represent arithmetical averages of disease indices obtained from duplicate tests at each temperature. At 18°, Wisconsin Perfection inoculated by the clip method showed a more rapid and more severe disease development than when inoculated by the dip method. Near-wilt resistant Delwiche Commando in both inoculated and check rows, and Wisconsin Perfection in check rows, showed a much lower and nearly similar amount of disease development. It may be seen in figure 2 that the Wisconsin Perfection seedlings in rows 4 and 8 inoculated by the clip method were more severely diseased at 20 days than those in row 2 inoculated by the dip method. However, the differential between the resistant and susceptible types of reaction at this temperature in this length of time was not so great as was desired.

At 21° the rates of disease development were in much the same relative order as at 18°, though they were generally more rapid and advanced at the end of 20 days. Wisconsin Perfection seedlings inoculated by the clip method had almost completely succumbed to the disease in from 15 to 20 days following inoculation, while resistant Delwiche Commando, in a like period of time, showed disease indices of 7 in 15 days to 30 in 20 days following inoculation. The clip method of inoculation was superior to the dip method for obtaining a differential for reaction to near-wilt at this temperature without a significant breakdown of resistance of Delwiche Commando. The comparison is evident in figure 2, where rows 3 and 4, 7 and 8, inoculated by clipping, show a better disease reaction differential than rows

1 and 2, inoculated by dipping. The control rows appeared little affected by the clipping treatment using distilled water.

At 24° the rates of disease development retained the same relative order as at 21°, although they were more rapid. Wisconsin Perfection seedlings inoculated by the clip method gave, at 18 days after inoculation, an average disease index of 100, while at 20 days those seedlings inoculated by the dip method did not exceed a disease index of 82. The resistance of Delwiche Commando, however, had begun to break down, since at 24°, by the clip method, it attained a disease index of 36, which was slightly higher than the index value at 21°.

At 27° C., the rates of disease development for the inoculated seedlings were considerably faster than at the lower temperatures. Disease indices for Delwiche Commando, for the clip and dip methods of inoculation, rose to 66 and 59 respectively after 20 days, indicating a continued decrease in disease resistance at higher temperatures. The appearance of Delwiche Commando plants at 27° C., 20 days after the two types of inoculation, is shown in figure 2. At this temperature the distinction between resistant and susceptible plants was obscured by the aggressive action of the pathogen.

In early experiments, seed coats were removed from the seed at time of inoculation. Decay of many of the seed coats provides a medium for the growth of the near-wilt organism and enables it to enter and rot the cotyledons. When this occurs it is difficult to distinguish between plants in the resistant and susceptible classes. An experiment was planned to determine whether seed protectants could be used in the near-wilt test to reduce seed decay before and after inoculation. The materials used were tetrachloroquinone (Spergon) and sodium hypochlorite (Clorox). A solution was prepared by mixing one part Clorox with 10 parts of water. Seeds were first immersed in a solution of Dreft, a commercial detergent, washed in two changes of distilled water, immersed in the Clorox solution for 10 min., and then washed free of the treatment solution with three changes of distilled water. For the Spergon treatment, seeds were shaken in a small bottle with a sufficient quantity of the protectant dust to completely cover them with an amount in excess of that used commercially.

Two 6-in. clay pots were provided for each treatment except the control. The drainage holes were covered with 1-in. square pieces of glass wool and the pots were filled with sterile white quartz sand to within about 1½ in. of the top. Six seeds of each variety were planted per pot, one variety on each side. Eight days after planting, the seedlings were removed and the seeds examined for decay. Seed coats were removed from half of the seeds in each treatment. Plants intended for inoculation were then given the clipping treatment as previously described. This experiment was conducted in an air temperature sufficiently high to maintain a sand temperature of 21° to 23° C.

Data from the seed treatment experiment are summarized in table 1.

During the 9 days after planting and preceding inoculation, none of the 46 seeds treated with Spergon showed decay of the seed coat. Seven seeds, however, had minor cotyledonary lesions apparently originating from invasions of fungi before the seed had been harvested. Clorox was less effective than Spergon, for 12 seeds out of 46 that were examined had decaying seed coats and 3 had minor cotyledonary lesions. Twenty-seven out of 48

TABLE 1.—*Effects of seed treatments upon seed decay and disease index of near-wilt resistant Delwiche Commando and near wilt susceptible Wisconsin Perfection peas*

Treatment	Variety[a]	Type of seed infection at inoculation time		Total plants	Disease index after indicated days from date of inoculation	
		Decaying seed coats	Cotyledonary lesions		20	27
		Number	*Number*	*Number*		
Seed coats left on Inoculated						
Clorox	DC	0	0	12	45	78
	WP	3	0	10	93	100
Spergon	DC	0	1	12	4	13
	WP	0	0	12	73	94
Control	DC	6	0	12	14	55
	WP	8	0	12	94	100
Noninoculated	DC	1	2	4	0	0
	WP	2	2	6	0	0
Seed coats removed Inoculated						
Clorox	DC	4	1	12	11	25
	WP	5	2	12	77	94
Spergon	DC	0	3	11	9	9
	WP	0	3	11	63	88
Control	DC	4	0	12	17	36
	WP	9	1	12	88	100
Noninoculated	DC	3	1	6	0	0
	WP	3	0	5	0	0

[a] DC = Delwiche Commando; WP = Wisconsin Perfection

untreated seeds had decaying seed coats and only one of these had a cotyledonary lesion. It was clear that Spergon effectively protected the pea seeds from attacks by fungi during the period from planting to time of inoculation.

Disease indices of 78 and 55 at 27 days were obtained for Delwiche Commando, treated with Clorox and in the control, respectively, in comparison with the much lower disease index of 13 for Commando given the Spergon treatment. Spergon prevented the resistance breakdown that results from invasion of the cotyledonary region by the near-wilt fungus following inoculation when the seed coats are not removed. Further evidence for the role of the fungus in attacking unprotected cotyledons and inciting disease is

the fact that no near-wilt developed in noninoculated Commando plants produced from seeds with seed coats in place, even though such seedlings had signs of decay 8 days after planting. The susceptible Wisconsin Perfection grown from Spergon-treated seed gave an index of 94 which was six points lower than for the other two treatments in the series where seed coats were left intact. The greater differential between the two varieties in the Spergon treatment appeared to be adequate in tests for reaction to near-wilt.

Table 1 also shows disease development indices for the three seed treatments when seed coats were removed from the seed at time of inoculation. Indices for the Spergon and check treatments are of primary interest. For the Spergon treatment the indices were 9 and 88 for the two varieties, a differential about the same as that in the series where seed coats were not removed.

An idea of the effectiveness of the near-wilt inoculation procedure accompanied by seed treatment can be gained from a sample of the results obtained in actual breeding studies. Forty-six F_3 lines harvested in bulk in 1947 were tested in the F_4 generation. This cross had been tested in F_2 in the field in 1946 and the susceptible plants discarded. In the greenhouse test, 29 of the lines were classified as segregating and 17 as resistant. This was a very close approach to the expected 2:1 ratio.

DISCUSSION AND SUMMARY

The object of the investigation was to develop a standardized technique whereby breeding material could be tested in the greenhouse for reaction to the near-wilt disease, incited by *Fusarium oxysporum* f. *pisi* (Linford) race 2 Snyder and Hansen. The experiments demonstrated that uniform and consistent results could be obtained by planting seedlings in clean quartz sand and inoculating 7 to 10 days later by cutting the roots about 1 in. below the seed while they were immersed in a spore and mycelial-fragment suspension; 18 to 20 days later an adequate index of reaction to near-wilt was obtained. This test was more rapid than when peas were planted in the greenhouse in field soil infested with the near-wilt organism. Under the latter conditions it was shown by Virgin and Walker (4) that severe disease development occurred only after 52 days from the time of planting. The former test was more dependable than disease reaction trials made in the field where environmental conditions were often unfavorable for the complete elimination of susceptible plants.

A sand temperature of 21° C. maintained throughout the period of testing was found to provide the best differential between the resistant and susceptible varieties. At 24° the resistant variety showed greater disease development, while at 27° the breakdown of resistance was still more pronounced. At 18°, disease development was so slow that after 18 to 20 days it was not possible to differentiate accurately between resistant and susceptible plants.

It was shown that if seed is treated with Spergon before planting it is unnecessary to remove the seed coats at the time of inoculation.

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EFFECT OF SOIL MOISTURE AND MINERAL NUTRIENT CONCENTRATION ON THE DEVELOPMENT OF BEAN POWDERY MILDEW

C. E. YARWOOD

The observation that some powdery mildews develop more luxuriantly in dry seasons and situations than under relatively wet conditions has been recorded by a competent authority as early as 1864 (1). In more recent times Cook (3) observed that bean mildew (*Erysiphe polygoni* DC) developed luxuriantly on plants in dry soil, and Volk (10) showed, in controlled soil moisture tests, that *Erysiphe graminis* developed more and caused less host necrosis on rye grown in soil at 40 per cent of its water-holding capacity than in soil at 60 or 80 per cent of its water-holding capacity. Nevertheless, much contrary opinion exists, and a recent plant pathology text states that powdery mildew of cereals "is most severe under moist conditions and those favoring vigorous, luxuriant growth of the host" (2). So far as the writer is aware no quantitative information on the effect of soil moisture on the injury caused by any powdery mildew has been presented, and this report on the subject may therefore be of value.

METHODS

For soils in which plants are growing there is no satisfactory method of maintaining soil moisture at uniform levels below field capacity (6). When an attempt is made to wet a soil mass to some moisture content less than field capacity, a portion of the soil is wetted to field capacity and the remainder is not penetrated, or penetrated very slowly, by the added moisture. Therefore problems of the type described here, and similar ones, might be considered insoluble by conventional methods. Nevertheless, conditions resulting from varying additions of water below the field capacity of the root-bearing mass bring about changes in plant growth and in plant disease development which are economically important and worthy of description, and seem most appropriately ascribed to soil moisture.

For soil moisture studies a uniform group of 5-in. pots was tared with well-mixed loam soil, planted with 5 Pinto bean seeds per pot, and watered uniformly until the plants were up, about 7 days after seeding. The plants were thinned to 2 per pot, the gross weight of the pots for moisture saturation to field capacity was determined, and the pots were divided into 2 to 5 groups for varying soil moisture conditions from field capacity to a little above the permanent wilting point. Enough water was added to the soil surface of each pot each morning to bring the gross weight to that arbitrarily decided upon. For each soil moisture condition one set of plants was inoculated with powdery mildew by spraying both leaf surfaces with a sus-

pension of conidia in water, and another set was maintained as a control. If controls were maintained longer than 2 weeks it was necessary to destroy on them the mildew that resulted from natural infection. For this purpose 0.1 per cent lime sulphur solution was sprayed on the plants. This treatment had little effect on the growth of non-mildewed plants. At the conclusion of the test, soil moisture samples were taken and the plants were harvested. One soil sample for each moisture condition was taken in the morning before the pots were tared with their daily addition of water, and another after. Samples of about 20 grams, taken after the entire soil mass in a given pot was well mixed, were dried in an oven for 3 days at 90° C. and weighed. The moisture present was designated in percentage by weight of the oven dry weight of the soil. In this way the approximate maximum daily range in soil moisture for each moisture condition was determined for most trials. An attempt was made to determine the permanent wilting point of the soil in one trial. Six pots of bean plants grown under conditions of regular and adequate watering were held without water for 3 days, and all plants were severely wilted. When pots were placed in a moist chamber, plants in 3 of the pots recovered their turgor in 24 hr. but plants in 3 pots remained wilted. Soil samples contained 7.3 to 11 per cent moisture where recovery occurred and 4.7 to 8.6 per cent where plants were permanently wilted. The permanent wilting point of this soil, therefore, was close to 7 per cent, which was about 25 per cent of the water-holding capacity. This wilting point is considerably above the lowest soil moisture recorded for plants growing in the driest soil. Soil samples were taken after the soil was mixed and the recorded soil moisture in the low soil moisture series would be the average of the air-dry soil in the bottom of the pot and the smaller amount of moist soil in the upper part of the pot.

For studies of the effect of nutrient concentration on the development of bean mildew, Pinto beans were seeded in sand and later transferred to 300-cc. flasks of water or Hoagland's solution (5).

For green weight yield, all leaves were weighed, and in some trials the weight of stems beyond the primary leaves was included because this stem growth had formed after treatments were begun. Time of harvest varied in order to secure the greatest effects of mildew infection and of soil moisture or nutrient treatment in the shortest time, but was usually about 6 weeks after seeding.

Reduction in yield due to mildew infection was calculated as the difference between the green weight of the healthy and mildewed plants expressed as a percentage of the green weight of the healthy. This method indicates much greater yield differences due to treatment than would be apparent from absolute values. For example, in the data presented in figure 1, the absolute reductions in yield due to mildew infection at low and high soil moisture were 1.50 and 1.47 gm., respectively, an insignifi-

cant difference, while the percentage reductions were 81 and 39, respectively, a highly significant difference.

Mildew development was rated on an arbitrary scale of 0 to 10 in which 0 indicated no mildew apparent, and 10 indicated that the leaf was entirely covered with a vigorous growth of mildew. In some trials mildew infection was measured quantitatively as conidia produced per unit area per day, but this method was not very satisfactory. The day prior to such an examination most of the conidia were removed by shaking the leaves. The next day slides were pressed against typical mildewed areas and the number of conidia per low-power field of the microscope was counted. Since the efficiency of the method was not tested, it is likely that the number of conidia found was less by some undetermined amount than the number actually present. Host necrosis due to mildew infection was rated on an arbitrary scale of 0 to 10 in which 0 indicated no necrosis apparent to the unaided eye and 10 indicated that a great majority of epidermal cells of upper leaf surface were necrotic. Host necrosis following powdery mildew inoculation is a common manifestation of resistance.

EXPERIMENTAL RESULTS

Results of a typical trial of the effect of soil moisture on yield and mildew severity are summarized in part in figure 1. There were 2 plants per
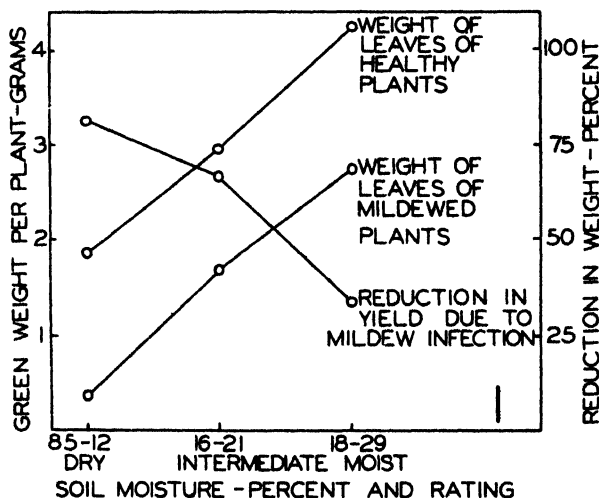
FIG. 1. Effect of soil moisture on the green weight of leaves from healthy and mildewed plants, in one trial started in November and harvested December 18, 1946.

pot and 4 pot replications of each treatment. The seed was sown on November 9, 1946, soil moisture adjustments at 3 soil moisture levels were started November 19, half the plants were inoculated November 27, and the plants were harvested and soil moisture samples taken December 18. Plant growth increased greatly with increasing soil moisture. The green

weight per plant of healthy plants was 1.83 gm. at low soil moisture, 2.96 gm. per plant at intermediate soil moisture, and 4.22 gm. per plant at high soil moisture. Mildew developed luxuriantly and without causing host necrosis on the small dark green primary leaves formed at low soil moisture; it was progressively poorer and host necrosis was extensive on the large pale leaves formed at intermediate and high soil moisture. Reduction in yield due to mildew infection decreased with increasing soil moisture, being 82 per cent in dry soil at 8.5 to 12 per cent moisture, 67 per cent in soil at 16 to 21 per cent moisture, and 39 per cent in soil at 18 to 29 per cent soil moisture, when the water-holding capacity of the soil was 29 per cent of its oven-dry weight.

Average results of all 16 trials of the effect of soil moisture on bean powdery mildew are summarized in figure 2. The number of soil moisture
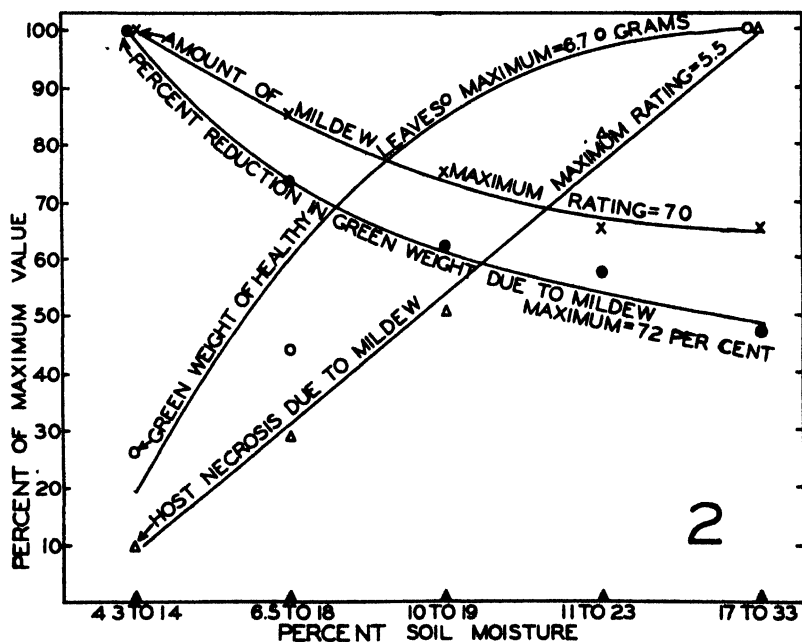
FIG. 2. Effect of soil moisture on the green weight of leaves from healthy plants, on the amount of mildew on inoculated plants, on the reduction in green weight due to mildew infection, and on host necrosis due to mildew infection.

gradations, the number of replications, the age of the plants at the time of soil moisture adjustments, the dosage of inoculum, the time of year, the age of infection at time of recording results, and the completeness of the record, all varied in different trials and are believed to have an important though inadequately determined bearing on the results. In other words, the slopes of the curves in figure 2 would be and were greatly different under different conditions. For example, the amount of mildew and host necrosis was greatly affected by the amount of inoculum and age of the leaves at time of inoculation. Although infection has been secured from

single conidia, infection resulting from scanty inoculum was relatively weak and necrosis due to mildew was relatively severe on leaves grown at high soil moisture. If, on the other hand, leaves were inoculated heavily while young, the difference in mildew development due to soil moisture was relatively slight. Such differences, as well as those due to nutrient concentration, were greater on primary bean leaves than on secondary bean leaves.

At time of harvest the bean roots had extended throughout the 5-in. depth of the soil layer, and even in the low moisture series where the lower layers of soil were air-dry these lower roots were still turgid, indicating that they might still be functioning and that they were receiving moisture from the regularly watered roots above. It is not known what bearing, if any, these turgid roots in dry soil had on mildew development.
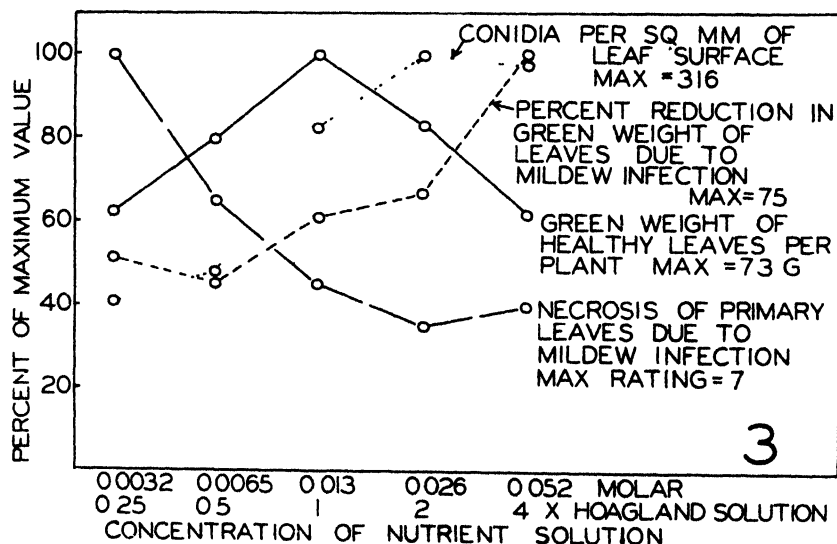
FIG. 3. Effect of concentration of nutrient solution on green weight of leaves of healthy plants, on the reduction in green weight due to mildew infection, on the production of conidia, and on host necrosis due to mildew infection.

The plants growing in soil at the lowest moisture content frequently wilted during the day and remained wilted until the regular watering next morning. This wilting did not seem to be detrimental to the mildew growing on these leaves, and may perhaps have favored it, though this was not studied. Plants growing in the next to the lowest soil moisture condition (6.5 to 18 per cent) also wilted on bright days. Plants at the 10 to 19 per cent soil moisture level were never observed to wilt, yet they yielded less than plants at higher soil moisture, indicating that soil moisture levels below field capacity but above the wilting point influenced plant growth.

Results of three trials to determine the effect of mineral nutrient concentration on the development of bean powdery mildew are presented in figures 3 and 4. Increasing or decreasing the nutrient concentration in the

Hoagland formula decreased the growth of beans. Mildew development and reduction in yield due to mildew increased with increasing nutrient concentration while necrosis due to mildew decreased. In two other trials mildew severity on the primary leaves of plants grown in distilled water and 0.1, 0.2, and 10 times the concentration of nutrients in the Hoagland

FIG. 4. Effect of nutrient concentration on the development of powdery mildew on primary bean leaves. From left to right the primary leaves are from plants growing in 0.25, 0.5, 1, 2, and 4 times the concentration of nutrients in Hoagland's formula. Mildew development is progressively greater, and host necrosis due to mildew is progressively less from left to right.

formula showed a similar relation, but these nutrient conditions were all beyond the limits for good growth of beans. These results with bean mildew appear in accord with those of Grainger (4) for oat mildew.

From plants for these trials grown in water culture and in soil, data on the dry matter content of the leaves and on nodulation of the roots were secured and average results are presented in table 1. In both water and

TABLE 1.—*Effect of cultural and infection conditions on dry matter content of leaves and nodulation of roots*

Concentration of nutrient solution or soil moisture content	Percentage dry matter of leaves		Root nodules per plant	
	Healthy	Mildewed	Healthy	Mildewed
A. Plants in water culture				
0.0032 molar	13.0	13.5	59	28
0.0065	12.5	13.0	16	8
0.013	11.5	11.9	9	2
0.026	12.7	13.1	2	0
0.052	15.5	16.9	0	0
B. Plants in soil				
10.2 per cent soil moisture	10.4	11.3		
18.5	8.5	9.5		
23.5	7.5	9.2		

soil cultures mildewed leaves always had a greater percentage of dry matter than healthy leaves. The mildewed plants always yielded less than the healthy (Figs. 1, 2, 3). The percentage of dry matter of healthy leaves from plants grown in water culture decreased with increasing nutrient con-

centration up to 0.013 M and then increased up to 0.052. Plant growth was greatest at 0.013 M (Fig. 3). With plants in soil the percentage of dry matter of healthy leaves increased from 7.5 to 10.4 per cent as soil moisture decreased from 23.5 to 10.2 per cent. Plant growth was greatest at 23.5 per cent (Fig. 1, 2). Therefore with respect to infected *vs.* healthy plants, low *vs.* high concentration of nutrient solution, and low *vs.* high soil moisture content there was a positive correlation between high moisture content of the leaves and growth vigor of the plants.

Nodulation of roots decreased with increasing nutrient concentration and was always lower for mildewed than for healthy plants (Table 1).

OBSERVATIONS ON OTHER POWDERY MILDEWS

In addition to the studies of bean powdery mildew already reported, the effect of soil moisture was studied for the powdery mildews of red clover (*Erysiphe polygoni*), pea (*E. polygoni*), mustard (*E. polygoni*), barley (*E. graminis*), cucumber (*E. cichoracearum*), sunflower (*E. cichoracearum*), rose (*Sphaerotheca pannosa*), and grape (*Uncinula necator*) in the greenhouse at Berkeley, California, and for the powdery mildews of bean, barley, cucumber, sunflower, and rose in the field. Of these powdery mildews, all with the exception of grape powdery mildew developed better on plants at low soil moisture than on plants at high soil moisture, but the effect was pronounced only with bean and with mustard. With beans the varieties Pinto, Scarlet Runner, and Dwarf Lima were used in one parallel test and the favorable effect of low soil moisture on mildew development was most pronounced on Pinto. With barley the effect of soil moisture on mildew development was tested for the varieties Atlas, Black Hulless, Kwan, Algerian, and Psakwan, a series ranging from high susceptibility to high resistance. It was thought that low soil moisture might greatly lower the genetic resistance of the resistant varieties, but such was not the case. The apparent greater mildew infection on grape at high soil moisture than at low soil moisture was slight in the only test performed and is not considered significant.

DISCUSSION

The basic reason why beans and some other plants grown under conditions of low soil moisture or high nutrient concentration are more susceptible to powdery mildew than plants grown under conditions of high soil moisture or low nutrient concentration is not known. The writer believes that both situations may be related to the xerophytic nature of powdery mildews (11), since conditions which favor mildew development are those producing a xerophytic environment for the plant. Here, in contrast to the previously described situation (11), however, the region of treatment—*i.e.*, the roots, is far removed from the region of response, the leaves. The greater water content of leaves from plants grown in 0.013 M nutrient solution than of leaves from plants grown at lower or higher nu-

trient concentration, while mildew increased progressively from 0.0032 to 0.052 M, would not support this point of view. Of course there is a nice correlation between supply of nutrients to the host and mildew development, and this may be the proper explanation. Opposed to this argument is the fact that increasing nutrient concentrations beyond 0.013 M which increased mildew development did not increase plant growth.

Although the opinion popularly prevails that powdery mildews, like obligate parasites in general, develop more luxuriantly on a vigorous than on a weak host (7), little support for it is found in this study. Leaves on plants grown at low soil moisture were much darker green and more susceptible to mildew than leaves of plants grown at high soil moisture. However, for plants in soil there was an inverse relation between mildew development and host growth (see figure 2), and for plants in water culture there was a direct relation between mildew development and plant growth below 0.013 molar nutrient concentration, but an inverse relation above 0.013 molar. A similar lack of correlation between wheat growth and wheat mildew development has been reported by Trelease and Trelease (9) in studies of the relation of mineral nutrition of the host to mildew development.

The possibility that the treatments to which the plants were subjected affected the relative humidity at the leaf surfaces, which in turn affected mildew development, is considered unlikely though the effects are partly in accord with the idea that powdery mildews may be favored by low atmospheric humidity (11). Perhaps the expected lower turgor pressure of the leaves on plants grown at low soil moisture or high nutrient concentration is involved, since Rivera (8) has reported that decreased turgor increased the susceptibility of wheat to powdery mildew.

SUMMARY

On Pinto bean plants grown in a series of soil moisture levels from 4 to 33 per cent of the dry weight of the soil, mildew developed more luxuriantly, host necrosis due to mildew was less, and reduction in green weight due to mildew infection was greater at low than at high soil moisture. Powdery mildews of red clover, pea, mustard, barley, cucumber, sunflower, and rose in greenhouse and field trials also developed more luxuriantly on plants grown at low than at high soil moisture, though quantitative results were not taken in most cases. On beans grown in water culture with nutrient concentrations ranging from 0 to 10 times the concentration of nutrients in Hoagland's solution, mildew development increased, host necrosis due to mildew decreased, and the effect of mildew on green weight increased with increasing nutrient concentration, while plant growth was greatest at the standard concentration.

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LIFE HISTORY AND CONTROL OF THE ASCOCHYTA RAY BLIGHT OF CHRYSANTHEMUM

KENNETH F. BAKER, A. W. DIMOCK, AND LILY H. DAVIS[1]

The Ascochyta ray blight of chrysanthemum (*Chrysanthemum morifolium* Ram.) was first observed by F. L. Stevens outdoors and under glass in two counties of North Carolina during November, 1906, and the pathogenicity of the fungus, *Ascochyta chrysanthemi* Stev., was established (16). The disease apparently had been known there since 1904. According to records of the Plant Disease Survey,[2] it was reported there again by Stevens in 1907 and by H. R. Fulton in 1913. In South Carolina it was reported by H. W. Barre in 1912 and 1913 and by R. F. Poole in 1931. In Mississippi it was reported by L. E. Miles in 1932 (21). The disease occurred in outdoor plantings in Maryland in 1947 and was severe in 1948, causing typical flower blight as well as stem cankers.[3] The occurrence of the disease in Florida has recently been confirmed by us. Apparently the fungus has continued to be destructive in the area of its first recognition in North Carolina, and caused severe losses in some field and cloth-house plantings in 1946 and 1947.

H. N. Hansen and W. C. Snyder observed Ascochyta pycnidia and Mycosphaerella perithecia on dead stem bases of commercial chrysanthemums from San Mateo county in March, 1949. Single-ascospore and single-conidium cultures proved these to be the same fungus. Single-conidium cultures grown on plain agar plus cold-sterilized pea stems developed mature perithecia in 10 days outdoors. Flower isolates produced typical petal spots which then advanced into ray blight. These results with the California isolate have been confirmed by us at Los Angeles. Pycnidia and perithecia developed on petals of flowers exposed under moist outdoor conditions for 13 days. The disease has never been reported in California and this record is therefore of unusual interest since it shows that the fungus will persist in an area of unfavorable climate where the natural flower infections are not known to occur.

The fungus was mentioned in publications from Ohio (13, 19), Missouri (12), New Jersey (11), New York (3, 7), Wisconsin (1), Colorado (4), and Germany (10), apparently based on literature review and without any actual evidence of its occurrence in these areas.

FIG. 1. Ascochyta ray blight of chrysanthemum. A, naturally infected flowers (Pittsboro, North Carolina, September, 1947) showing characteristic unilateral infections. B, experimental conidial infections on moderately susceptible Goldsmith after 96 hr. Enlarged petals below. C, same on very susceptible Arcadia. Detached petals half-rotted. Photographs by W. R. Fisher, Department of Plant Pathology, Cornell University.

The present studies were conducted to clarify some points in the life history of the fungus, to determine whether there was danger that it might become established in other areas, and to devise control procedures for the disease produced.

SYMPTOMS

Symptoms in naturally infected material were found to agree with those described and illustrated by Stevens (16). The disease is first noted and is most important in its attack on the ray flowers of the head. Infection usually is initiated on one side of the blossom but may later spread until all the florets are involved (Fig. 1, A). Growth of the fungus char-

FIG. 2. Sporulation of Ascochyta ray blight fungus on chrysanthemum. A, enlarged Ascochyta pycnidia on petals of flowers from Pittsboro, North Carolina, September, 1947 (×5). B, enlarged perithecia on flower inoculated with conidia (×4). C, enlarged perithecia on stem of naturally infected material held in the laboratory (×16). Photographs by W. R. Fisher, Department of Plant Pathology, Cornell University.

acteristically causes tissue discoloration of the floret progressively upward from the receptacle. The affected petals turn tan to light brown and remain so except when invaded by secondary organisms. In many, perhaps most, cases the fungus grows down into the peduncle for several centimeters, causing it to turn black and weaken, and the head to droop. The

fungus frequently attacks unopened buds and their peduncles, darkening the bracts and stem tissue.

Leaf infection is fairly common on plants having diseased flowers, producing in the lamina irregular black blotches up to 2–3 cm. across. Black girdling lesions several centimeters long frequently are noted on the stems, usually starting at a node.

Pycnidia were abundant in all affected tissues of most field infections seen by us, particularly in the petals (Fig. 2, A). These structures, amber-colored at first but later darkening, were observed readily with a 15 × hand lens. It also was noted in our field material that the infected florets, particularly those bearing pycnidia, were almost invariably stuck together. Whether the petals were bound together by the permeating hyphae or were cemented by the gelatinous material extruded with the spores was not determined, although the following observations suggest the latter.

Certain additional symptoms became evident on material inoculated with suspensions of either spores or mycelial fragments. and maintained under laboratory temperatures of 22°–24° C. The first evidence of infection was the appearance of abundant, minute, deep pink spots[4] on the petals of the test varieties Arcadia, Rev Bushnell, and Goldsmith (Fig. 2, B). Within a few hours the petals of Arcadia were badly rotted, becoming tan in color. Although overgrown with mycelium when kept under a bell jar, they remained distinct rather than becoming stuck together. Pycnidia were not formed in the laboratory but developed abundantly within a week after the material was exposed to the fluctuating temperature of the laboratory roof (Ithaca, N. Y., in April).

Leaves inoculated with suspensions of spores or mycelial fragments in the laboratory soon developed abundant, minute, black lesions. The surrounding green tissues yellowed very rapidly when the leaves were maintained in a moist chamber. Although the lesions enlarged somewhat, blotches similar in size to those on field material were not formed.

The disease may develop with dramatic rapidity under rainy conditions in the field, causing total loss of entire blocks of susceptible varieties which appeared perfectly healthy several days previously. Symptoms may appear also on apparently healthy cut blooms in transit to market, rendering them unfit for sale.

The disease is distinguished easily from all other chrysanthemum troubles Botrytis cinerea Pers. may cause tiny brown spots and finally produce decay of the flowers under cool humid conditions (15), but generally the characteristic gray sporulation occurs copiously on the petals. Usually Ascochyta-infected flowers are free from secondary fungi, and the pycnidia are conspicuously abundant. Septoria spp. are not known to attack chrysanthemum petals but are common on leaves, producing symp-

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[4] This symptom was also reported by a commercial grower as the first evidence of field infection.
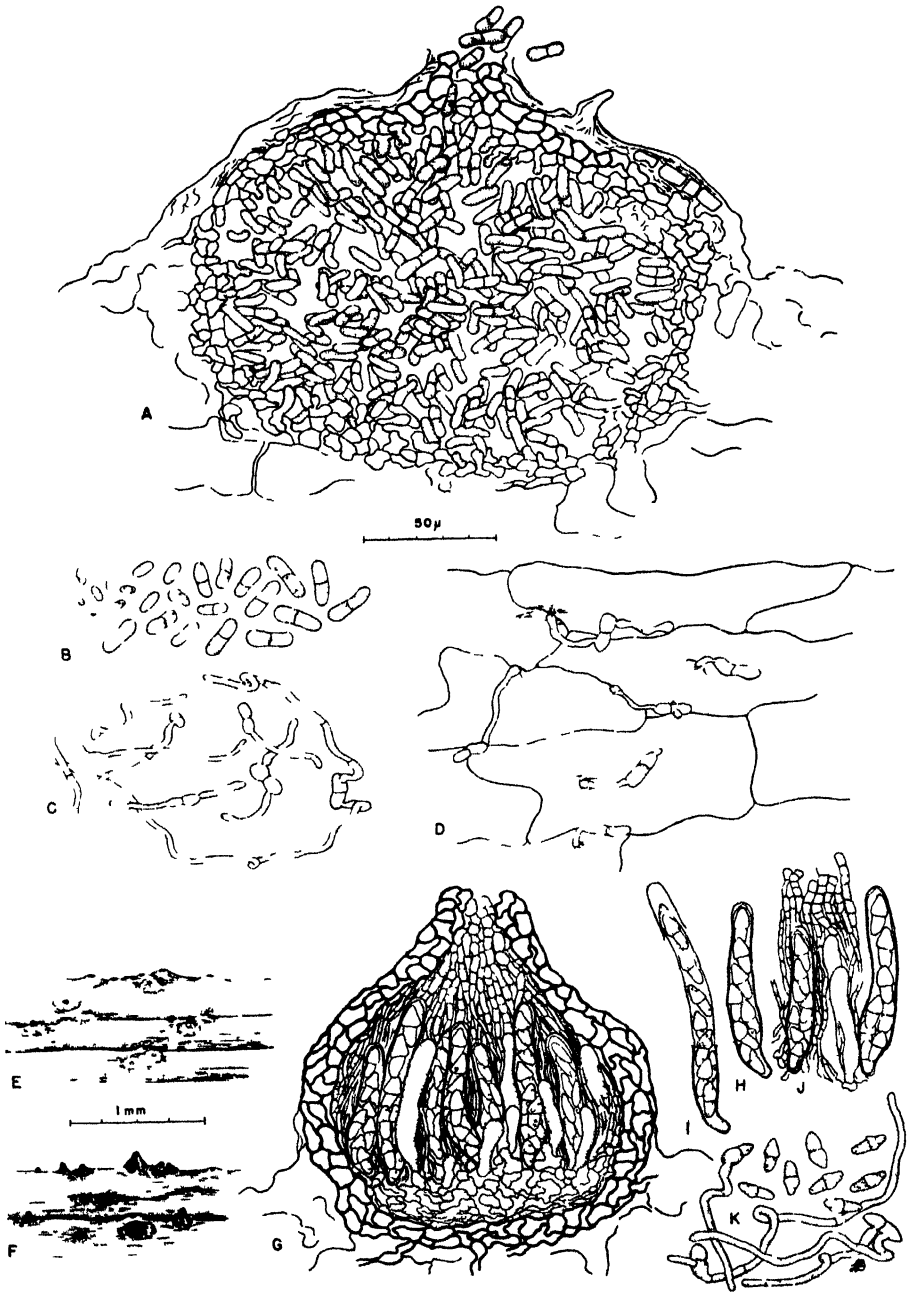
Fig. 3. Camera lucida drawings of *Mycosphaerella ligulicola*. Magnification of A–D and G–K on the 50 μ scale. A, cross section of pycnidium of Ascochyta stage. B, conidia, ranging from microspores to uniseptate type. C, germinating conidia on water agar after 18 hr. D, composite of several germinating conidia penetrating chrysanthemum petal, 7 to 23 hr. after inoculation. E, habit sketch of pycnidia on peduncle, showing oozing spores. F, habit sketch showing perithecia on stem. G, cross section of perithecium showing asci in various stages of maturity, and strands of interthecial tissue. H, mature ascus. I, elongated ascus, showing dehiscence. J, segment of asci and interthecial tissue. K, mature ascospores, and stages in germination after 15 hr. Drawings by L. H. Davis, Division of Plant Pathology, University of California, Los Angeles.

toms somewhat different from those caused occasionally by *Ascochyta*. The margins of Septoria spots are more definite than those of *Ascochyta*, and the central areas have a characteristic sheen. Leaf tissue surrounding Ascochyta spots turns yellow more commonly than does that surrounding Septoria spots. The pycnidia of *Ascochyta* usually are pale and inconspicuous, with a darkened ostiole from which spores exude in amber drops or short tendrils (Fig. 3, E); those of *Septoria* are conspicuous and black, and exude spores in long white cirri.

Because the most characteristic symptom is the blighting of the ray flowers, in which abundant pycnidia are produced, the most descriptive and appropriate common name for the disease is Ascochyta ray blight of chrysanthemum.

VARIETAL REACTIONS

Controlled tests of varietal susceptibility were not undertaken, but the following tentative ratings of varieties current in the trade were supplied by a commercial grower in North Carolina.

Resistant: Golden Herald, Hasagawa Gold, Irene, Minong, Nuggets, Red Daisy, Royal Crimson, Yellow Daisy, Yellow Irene.

Intermediate: Barcarole, Bittersweet, Bulkeley, Cameo, Capt. Cook, Carnelia, Golden Spray, Goldsmith,[5] Pink Dot, Pinocchio, Rev. Bushnell,[5]

[5] Confirmed in our laboratory tests.

Robin Hood, Valencia.

Very Susceptible: Arcadia,[5] Caroline Yosick, Cassandra, Gold Coast, Jessie, Jewell, Lakme, Mary Lennon Hall, Masterpiece, Navaho, October Bronze, Pink Pearl, Pixie, Princeton, Priscilla, Sea Gull, Shasta, Silver Sheen, Sunnyside, Sylvanna, Vesper, White Mensa, Yellow Dot, Yellow Hammer, Yellow Fellow, Yolanda.

Laboratory tests have shown Goldsmith to be somewhat less susceptible than Arcadia (Fig. 1, B and C), although still disastrously affected in the field.

The older varieties Nellie Pockett and Golden Wedding were noted by Stevens (16) as very susceptible.

Flowers and leaves of *Chrysanthemum leucanthemum* L. were not successfully infected in several attempts at Ithaca, N. Y., and Los Angeles, Calif. Because this plant commonly grows as an escape near the Pittsboro, North Carolina fields, it was considered a possible additional host of the fungus.

INFECTION STUDIES

Infections were successful in every attempt, whether with conidia from infected tissue or cultures, mycelial fragments from nonsporulating cultures fragmented in a Waring Blendor, or ascospores from dead stems. Flowers of a very susceptible variety heavily inoculated (*e.g.*, Arcadia dipped in a dense conidial suspension) and kept moist under bell jars were completely rotted in 15 hr. Check blossoms dipped in clear water

remained healthy even though retained under bell jars for many days. Previously (16), infection had been demonstrated only with macerated cultures of the pathogen.

Time required for infection. Germination of conidia occurred on agar in 3½ to 4 hr., but petal infection was found to require somewhat more than 6 hr. Flowers of Arcadia were atomized with a heavy suspension of conidia from field-infected flowers, and placed under bell jars. At intervals up to 6 hr. some flowers were removed and dried for 1 hr. in the air-stream of an electric fan blowing over a hot plate, then maintained uncovered and dry in the laboratory. Earlier tests had shown that established infections would continue without maintaining the blossoms at high humidity. No infection was evident in any of these lots after 5 days, although abundantly present after 3 days on check flowers kept moist under a bell jar. When subsequently atomized with clear water and replaced under bell jars, all test lots developed infection, indicating that conidia may start germination, be checked by drying, and resume growth when wetted as much as 5 days later.

Temperature relations. The effect of temperature on the rate of petal infection was studied. Flowers of Arcadia were placed under bell jars in temperature cabinets for 24 hr., removed briefly to be atomized with a conidial suspension prepared from field-infected flowers, and immediately replaced in the chambers. Observations made 19 hr. after inoculation revealed a broad temperature range suitable for infection, extending from 9° to 26° C., as indicated by the presence of characteristic pink spots in the petals. After 24 hr., infection was observed at 30° C., and after 42 hr. very tiny spots were seen at 6° C. Thus petal infection occurred at 6°–30° C., with the 20°–26° C. range highly favorable, and the optimum at about 24° C. The lesions at 6° C. were barely visible even after 42 hr. The pink color reaction of this white variety was evident at 6°–30° C. The relative growth rate of the spots at the various temperatures paralleled the amount of infection which had occurred. No infections occurred at 38° C. and the flowers remained in good condition for several days. Some of these removed to laboratory temperatures after 42 hr. developed scattering typical pink spots in 2 days, indicating the survival of a low percentage of the conidia. The checks were not infected.

The ability of the fungus to withstand extremely low temperatures was shown by overwintering tests in which field-infected material was placed in wire baskets on the laboratory roof (Ithaca, N. Y.) on October 20, 1947. Conidia obtained from this material on March 24, 1948, were viable and produced abundant flower infection after this exposure to an extremely cold winter with official temperatures at a near-by weather station reaching −29° C. No ascospores were involved in this carryover. Similarly, material overwintered at Pittsboro, North Carolina, yielded viable conidia.

The fungus grew well on potato-dextrose agar at a wide range of labo-

ratory temperatures. The optimum was about 24° C., with excellent growth from 15° to 27° C., and fair growth at 6° C.

Manner of infection. Conidia germinate on the flower petals and develop short mycelia before penetrating directly through or between epidermal cells (Fig. 3, D). The characteristic much-branched, short-celled mycelium (16) quickly grows through the tissue intra- and intercellularly, causing a moist brown decay.

THE CAUSAL FUNGUS

The fungus investigated unquestionably was the same as that studied by Stevens (16), even though minor morphological variations were observed. The collections studied were from Pittsboro, North Carolina, about 50 miles from the type locality and 30 miles from Stevens' Raleigh report. Pathogenicity was established repeatedly by fulfilling Koch's postulates.

Pycnidia are produced abundantly on the infected petals under conditions of fluctuating temperature and moisture (Fig. 2, A) but are only rarely formed in a continuously moist environment. They are somewhat less abundant on stems or leaves. In potato-dextrose-agar cultures pycnidia commonly are not formed, but some single-spore isolates produced them copiously. In culture, the pycnidia are brown and frequently coalesce so that more than one ostiole occurs. They are highly variable in size, as are the spores. The latter are also either 1- or 2-celled (Fig. 3, B). The reported 3-celled conidia (16) were not seen by us.

On the host the pycnidia arise on petals, leaves, or stems directly beneath the epidermis. As they enlarge, the epidermis is pushed up, but does not rupture except where the neck pierces it. At maturity the pycnidium is completely immersed except for the short-necked pore (Fig. 3, A). The wall is thin, only 1 or 2 layers, and amber in color except at the neck, where the cells are dark brown and thicker. In many cases the basal wall consists only of a thin (single-celled), translucent, proliferous stratum of mycelium. On the stems, the pycnidia are usually scattered and somewhat larger than on the petals where they are small and aggregated, perhaps because of the faster withering of the petals.

The internal cavity of the pycnidium is filled with a mass of hyaline spores budded from the fertile cells of the inner wall. The short sterigmata that Stevens (16) illustrated as bearing the conidia were not observed by us. At first the small continuous conidia were thought to be spermatia, but they were found just as capable of germination and infection as the larger 1-septate spores. On the host the conidia ooze in short columns (Fig. 3, E), but when the pycnidium is immersed in a drop of water, long twisting tendrils are formed. As the spore takes up water in germination the cells become distended and more or less globose, so that the spore becomes very deeply constricted at the septum without increasing appreciably in length. Germ tubes arise from one or both cells (Fig. 3, C).

Monoconidial isolates of the fungus on potato-dextrose agar at laboratory temperatures formed an appressed feathery growth 1.7 cm. in diameter after 67 hr.; in 4 days the colonies were round, 2.7 cm. in diameter, and the growth was cottony white; by 6 days, the colonies averaged 5.2 cm. in diameter, were salmon pink beneath (because of a diffusible pigment), and dark brown in the center. The margins were irregular, white, and appressed, but toward the center the colony was rather dense, floccose, and bluish gray, later darkening. Mature pycnidia were found in the center of colonies of sporulating isolates, but toward the margins only white mycelial knots were formed.

Perithecia are present in large numbers on infected chrysanthemum stems, petals, or leaves (Fig. 2, B and C). They were first observed January 12, 1948, on petals of infected flowers from North Carolina that had been kept dry in the laboratory at Ithaca, New York, for about 8 weeks. During that period the humidity was extremely low, and there was no possibility of the stems having been wetted. Some inoculated flowers that had been held in a moist chamber until decayed and then dried in the laboratory failed to develop pycnidia, but when examined after 6 to 8 weeks mature perithecia were found. This extraordinary development of perithecia under essentially arid conditions has been repeatedly confirmed. Overwintered infected stems collected at Pittsboro, North Carolina, on June 8, 1948, were held for herbarium specimens in Los Angeles, California. There were a very few perithecia on occasional stems at time of collection, but when the stems were examined again in October there were many grayish patches in which mature perithecia were closely crowded. A conidial suspension was placed on flowers in a moist chamber in the laboratory (Los Angeles) on August 26, 1948. Twelve days later abundant mature pycnidia were evident on the drying tissues, and by the 18th day a few immature perithecia were noted on the dry stems. No record has been found of a fungus of this type producing its perithecia under such arid conditions, but similar maturation of the perithecia in the fall months has been observed (17, pp. 581–582, 584). Jones (6) reported production of mature perithecia of *Mycosphaerella pinodes* (Berk. and Blox.) Stone on living plants 13 days after inoculation.

Perithecia formed, but much less abundantly, in naturally infected stems and flowers exposed outdoors (Ithaca) during the winter. Such material was placed in metal baskets on the ground on October 20, 1947. By February 23, 1948, immature perithecia were present. During the period October 20–November 23, 1947, in which the daily maximum temperature on 28 days reached or exceeded 6° C. (the approximate minimum for growth of the fungus), the mean minimum was 1.1° C. and the mean maximum 12.2° C. From November 24, 1947 to February 23, 1948, when only 7 scattered days reached or exceeded 6° C., the mean minimum temperature was −11.2° C. and the mean maximum was −1.0° C.

The perithecia were shown to be part of the life cycle of the *Ascochyta* by culture and inoculation tests. Single-ascospore cultures gave the same fungus as that obtained from conidia. The particular isolates thus obtained did not produce pycnidia in culture, but when one of the cultures was fragmented in a Waring Blendor and atomized on blossoms of Arcadia chrysanthemums, typical spots and decay developed in which pycnidia were formed when exposed outdoors (Ithaca) for 3 days in early April. These flowers were then kept dry in the laboratory for 50 days and typical mature perithecia were produced (Fig. 2, C) in stems and leaves. This behavior and the corroborative evidence of Hansen and Snyder already mentioned indicate that this fungus is homothallic. In another test single perithecia were removed from stems that had developed no pycnidia, and were crushed in sterile water to give a suspension of asci and ascospores. When drops of this were placed on chrysanthemum flowers, typical spots and decay developed within a few days.

The perithecial initials apparently develop just beneath the epidermis as small stromatal knots of mycelium. These enlarge until they break through the epidermis, becoming so erumpent in some instances as to appear almost superficial, but are basally adnate (Fig. 3, F). The centrum is at first filled with a hyaline pseudoparenchymatous mass of tissue near the base of which a fascicle of asci develops. Typically, in most species of *Mycosphaerella*, the pseudoparenchyma is crushed and disintegrated as the asci grow up into it, but in this case it is only partially destroyed, the bulk of it remaining as interthecial tissue in the mature perithecium (Fig. 3, G and J).[6] In this respect the fungus approaches the Dothioraceae in the higher Myriangiales, where each ascus grows up in a separate stromal locule, and differs from the Mycosphaerellaceae which usually lack threads in the uniloculate stroma (8).[7] Although atypical for *Mycosphaerella*, such mycelial strands have been noted by various workers for fungi included in the genus (5, 17).

At maturity the perithecium consists of an outer wall of dark brown, thick-walled cells in 2 to 3 layers and a central cavity composed of several layers of crushed pseudoparenchyma at the base, from which arises a cluster of asci, each apparently enclosed between fine mycelial strands which join in the neck of the perithecium (Fig. 3, G and J).

Not all of the asci mature at once, so that all stages of maturation are observable in one perithecium. The mature ascus (Fig. 3, H) is obclavate, gradually narrowed above, and abruptly contracted at the base, thus being

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[6] A special technique developed by L. H. Davis and J. G. Bald for rapid freehand sectioning under the wide-field microscope, and for prompt definitive staining of the sections has greatly facilitated these observations.

[7] Letter of July 24, 1948, from Dr. Julian H. Miller. After examination of a specimen of the chrysanthemum fungus, he classed it as an atypical *Mycosphaerella*. This concept is further discussed in the following paper: Miller, J. H. A revision of the classification of the Ascomycetes with special emphasis on the Pyrenomycetes. Mycologia 41: 99–127. 1949.

somewhat stipitate. It contains 8 sub-biseriate ascospores. The wall is double, consisting of a thick, fairly rigid outer, and a thin, elastic inner membrane. When the ascus is sufficiently mature, it ruptures at a thin place in the middle of the ectoascus, thus releasing the endoascus which elongates and projects into the neck of the perithecium. The ectoascus sometimes remains as a thimble-like cap at the tip of the endoascus (Fig. 3, I). Although forcible discharge of ascospores was not observed, the structure and rupture of the ascus wall and the compression of the ascospores into a single row are evidence for this type of discharge. The ascospores germinate readily, each cell sending out a germ tube (Fig. 3, K).

Only one *Mycosphaerella* or *Sphaerella* appears to have been described on chrysanthemum. *Sphaerella Chrysanthemi* F. Tassi was reported on dead stems of *C. marginata* in Italy in October, 1900 (18, p. 117). This description is so indefinite as to fit many species of *Mycosphaerella*. The perithecial stage reported here has little morphological distinctiveness and it is unlikely, therefore, that anything more definite could be determined even by examination of Tassi's specimens, if they could be found. It is impossible to determine whether Tassi's fungus was the same as the one considered here, and the fact that the disease is not known outside of the United States suggests that his fungus was different. The destructiveness of the disease under a wide range of environmental conditions is such that some record of it on the extensively cultivated chrysanthemum would inevitably have appeared if the fungus was present in Italy. Tassi's record affords no evidence of pathogenicity of his fungus. If the name *M. chrysanthemi* were to be assigned to our fungus there would be an unwarranted implication that the disease it produces occurs in Italy. Because of these facts our fungus is considered to be distinct. The *Ascochyta chrysanthemi* Cav. cited by Seymour (14, p. 681) was found to be based[3] on a brief note by Selby (13, p. 388) and probably represents an erroneous carryover of the authority from *Septoria chrysanthemi* Cav. mentioned 11 lines above on the same page.

The fungus is here placed in *Mycosphaerella* instead of *Sphaerella*, in accordance with present general usage. The Nomenclature Committee of the British Mycological Society (20, pp. 220–222) has recommended that the name *Sphaerella* (Fr.) Rabenh. be conserved for the genus, but this name apparently is a later homonym of, and still is used for, an algal genus (9, pp. 78–79).

Because of the points cited, the perithecial stage is considered as new, and is here named *Mycosphaerella ligulicola* n. sp. (from Latin *ligula*, little tongue [botanically, ray flower], and *colo*, to inhabit).

Mycosphaerella ligulicola sp. nov.

Peritheciis in caulibus, foliis et ligulis mortuis dispersis vel aggregatis, subepidermide erumpentibus, maculis pallidis griseis formantibus, nigris, globulosis, 96–224 μ

[3] Dr. W. L. White, of the Farlow Herbarium, kindly supplied this information from Seymour's notes in a letter of January 10, 1949.

diam. (medio 146 μ), ostiolo papilliformi, pariete membranaceo; ascis octosporis, fasciculatis, obclavatis, utrinque rotundatis, breve stipitatis, 49–81 × 8–10 μ (medio 63 × 9 μ); ascosporis subdistichis, elliptico-fusoideis, 1-septatis, loculis inaequalibus, ad septum constrictis, hyalinis vel pallide viridulis, guttulatis, 12–16 × 4–6 μ (medio 14 × 5 μ).

Perithecia numerous, crowded on grayish spots on old dried stems, less frequently on petals and leaves; erumpent, becoming nearly superficial, globose, 96–224 μ in diameter (average 146 μ), with a shiny, black, membranous wall and a distinctly papillate ostiole. Asci fasciculate, embedded in persistent mycelial strands which join together in the neck of the perithecium, ovoid-oblong, gradually narrowed above, short stipitate, 8-spored, 49-81 × 8–10 μ (average 63 × 9 μ). Ascospores hyaline to greenish, fusiform-elliptic, uniseptate and constricted, the upper cell abruptly swollen just above the septum, the lower cell narrower and acute, guttulate, subdistichous, 12–16 × 4–6 μ (average 14 × 5 μ).

In corollas, leaves, and stems of cultivated *Chrysanthemum morifolium* Ram. collected in Pittsboro, North Carolina, June 1948.

Pycnidia abundant, mostly scattered on stems, but densely gregarious on petals, covered by the epidermis except where the short-necked ostiole protrudes, depressed-globose, 111–325 μ in diameter on stems (average 182 μ), somewhat smaller on petals, 72–180 μ (average 122 μ), texture parenchymatous, the cells rather large and thin-walled, amber, dark brown around the ostiole. Spores ovoid to cylindrical, straight or somewhat curved, mostly becoming septate, hyaline, faintly guttulate, usually without constriction until germination, 8–13 × 3–4 μ (average 10 × 3 μ), the non-septate conidia being 4–10 × 2–4 μ (average 6 × 3 μ).

In corollas, stems, and petioles of cultivated *C. morifolium* Ram. causing blight, Fayetteville and Raleigh, North Carolina. *Ascochyta chrysanthemi* F. L. Stevens, Bot. Gaz. **44**: 241–258. 1907.

Apparently Stevens did not distribute exsiccati of *Ascochyta chrysanthemi* on the host, but sent out bits of dried culture with pycnidia. Consequently we have distributed specimens of both *A. chrysanthemi* and *Mycosphaerella ligulicola* on *Chrysanthemum morifolium* to the following herbaria: Department of Plant Pathology, Cornell University, Ithaca, N. Y.; Department of Botany, University of California, Berkeley; Division of Plant Pathology, University of California, Los Angeles; Farlow Herbarium, Harvard University, Cambridge, Mass.; U. S. Department of Agriculture, Beltsville, Md.; Commonwealth Mycological Institute, Kew, England; Department of Plant Pathology, University of Georgia, Athens.

Cultures of the fungus have been placed in the Centraalbureau voor Schimmelcultures, Baarn, Holland, and in the American Type Culture Collection, Washington, D. C.

LIFE HISTORY AND ENVIRONMENTAL RELATIONS

This fungus maintains a high biotic potential through most of the year in the region of its principal destructiveness, and seems to have similar

potentialities in other areas having frequent rains during the chrysanthemum growing season.

Pycnidia form readily and abundantly in infected flowers, buds, and peduncles, and to a less extent in stems and leaves, under field and clothhouse conditions. During wet periods enormous numbers of conidia exude in gelatinous drops or short cirri and are splashed or blown about in raindrops, but are not detached and spread by air currents alone. With the usual crowding of plants under cultural conditions it is possible for a single infection to spread to many near-by flowers. It is probable that the gelatinous spores get on the black cloth covers which are pulled over the plants each day and that, in wet periods, they are thus spread along the beds. Workmen also may spread the spores on clothing, tools, or hands. Such situations are known for Septoria leaf spot of this crop (2).

Because only a few hours are required for successful infection of the petals, short periods of rain are sufficient to induce the disease. The wide temperature range favorable for infection and growth (6°–30° C.) indicates that moisture would generally be more of a limiting factor to development of an epidemic. Once established, the fungus apparently can survive drying very well and resume growth in the tissue when rewetted. If it is well established, growth continues even under dry conditions. The current practice of growing plants in cloth houses under black cloth to regulate day length and thus control flowering tends to retard drying of the petals following a rain, and may favor production of a new crop of pycnidia within a few days. The number and rate of production of pycnidia makes it possible for a given focus of infection to enlarge and produce extensive loss in a short time.

The fungus is well adapted in several ways to survive the period between chrysanthemum crops. The pycnidia in stems and flowers are able to survive the winter and the enclosed spores are viable in the spring. The overwintering of perithecia has already been discussed and the point established that mycelia may overwinter in stems and later give rise to perithecia.

Development of the disease in the first flowers of each new season may, on the basis of our field observations in North Carolina, start in several ways. A few mature perithecia were present at Pittsboro on old stems on June 8, 1948, and, judging from the numbers of perithecia produced on such stems shipped to Los Angeles, California, they continued to be formed until late fall. In fact, when the fields were visited in late October, 1948, there was ample evidence of recent ascospore infection of the flowers. It is probable that ascospore discharge and infection occurs through much of the flowering season in the field. While observational evidence of forcible discharge of asci is lacking, the "jack-in-the-pulpit" type of dehiscence (Fig. 3, 1) is hardly one to release spores by any other method. The ascospores would be air-borne and would produce scattering

infections over the plants, as contrasted to the localized streaks of infection from water-borne conidia. It is not improbable, from the observed survival of the fungus, that it can overwinter as pycnidia on basal stems and leaves, and initiate infections in the spring by spores which splash upward, much as with Septoria leaf spot (2). Because of the infrequency of foliage or stem infections, this method of reaching the buds or flowers, often 3 feet above the ground, probably is not common.

The fungus commonly spreads into the peduncle from the infected corolla, and these bits of stem as well as other stem cankers are left in the field after harvesting. Ascospores produced on such stems may be carried by air currents to near-by fields of chrysanthemums.

That this fungus apparently did not spread out of the Southeastern States during a period of more than 40 years is difficult to explain. For the most part, propagative material has been shipped into but not out of this area, and spread thereby minimized. Until very recently almost no southeastern wholesale growers appear to have been shipping cut chrysanthemums into the Northern States. Recently, however, flowers have been extensively shipped, some of which must have been infected and hence dumped into rubbish piles. If such a pile was in the vicinity of flowering chrysanthemums, wind-borne ascospores might initiate infections and thus introduce the fungus to a new area. There apparently is nothing in the climate of the eastern, midwestern, and northwestern growing areas that would check the disease, once it was introduced. The dryness of the Southwestern States would seem to impose an effective limitation on the severity of the disease there if introduced.

The sequence of rainfall in the infested area in North Carolina may be important in the initiation of the perithecial stage. The mean monthly precipitation[9] of four stations in the area (Fayetteville, Raleigh, Durham, Greensboro) ranges from 3.41 to 3.80 in. from December through May, increases to 4.64–5.25 in. from June through August, then declines sharply to 3.63 in September, 2.72 in October, and 2.35 in November. The normal flowering months for chrysanthemum in that area are also the dry months in which perithecia would be most likely to mature. The development on the host under experimental conditions of immature perithecia in 13 days from actual infection indicates that their maturation and expulsion may occur within a relatively short time.

<div style="text-align:center">CONTROL</div>

Cultural practices. Use of planting stock from areas in which the disease occurs should be avoided since it appears that the pathogen might well become established in any region characterized by frequent rainfall during the growing season. Likewise, rotted flowers from known infected areas should be burned, rather than dumped on refuse piles.

[9] Climate and Man. U. S. Dept. Agr., Yearbook 1941: 1035–1037. 1941.

Practices designed to minimize the carryover of inoculum from one growing season to the next may be of considerable value. Fields should be plowed deeply in the fall to bury completely all residual plant material. All stems, leaves, *etc.* from cut flowers should be burned or buried, never dumped into a near-by ravine or rubbish pile. Crop rotation for at least 2 years is advisable. Propagation should be entirely by tip cuttings from stock plants grown under glass or other rainproof cover, and irrigated so as to preclude splashing water.

The use of waterproof bags tied over the buds before they begin to open was tested by one grower. About 95 per cent of the flowers so protected were free of disease, whereas almost all noncovered flowers were lost. This method of control might be practicable in small plantings or exhibition stock. Overhead sprinkling also should be avoided.

Under garden conditions, wider spacing and improved ventilation of plants would expedite drying and be of some benefit in reducing losses. The removal of the first infected buds early in the season would delay the increase of inoculum.

Fungicides. Since complete elimination of ray blight by cultural manipulation appears improbable due to the potentialities of the sexual stage and possible carryover on undetermined weed hosts, the value of protective sprays was investigated.

A preliminary experiment tested zinc ethylene-bis-dithiocarbamate (Parzate) at 1 lb. per 100 gal., dichloronaphthoquinone (Phygon) at $\frac{1}{2}$ and 1 lb. per 100 gal., phenyl mercury triethanol ammonium lactate (Puratized Agricultural Spray) at $\frac{1}{2}$ and 1 pint per 100 gal., with Tergitol No. 7 at 1–800 as the spreader in each case. All were completely effective in preventing infection, but severe petal injury occurred with Phygon and Puratized, and slight injury with Parzate or with Tergitol alone. Checks were heavily infected by the fungus.

A second experiment used Parzate at $\frac{1}{2}$ and 1 lb. per 100 gal. without spreader, and at 1 lb. per 100 gal. with Tergitol No. 7 at 1–2000, or DuPont 1N 3622 at 1–1000 and 1–2000. Complete protection without petal injury was obtained in each case, while heavy infection developed on the checks. The deposit was much less conspicuous when a spreader was used.

In these tests the fungicides were atomized on the blossoms and foliage of susceptible chrysanthemums (Arcadia, Rev. Bushnell, Goldsmith) and allowed to dry before being atomized with a conidial suspension taken from field-infected flowers. Each lot of flowers was then maintained under a separate bell jar at laboratory temperatures for 5 days, when records were taken.

While it is possible that Phygon and Puratized would be safe and effective at lower concentrations and that other fungicides also would be satisfactory, Parzate was recommended tentatively to growers on the basis of these tests. Reports from a North Carolina grower indicated successful

control during the 1948 season with Parzate at ¾ lb. per 100 gal. plus 6 oz. Lux soap (for spreader) and various insecticides applied every 7 days up to the time of flowering. Sprays were applied in the bud and blossom stage using Parzate at ⅜ lb. per 100 gal. plus 4 oz. Dreft detergent. Effective control might be obtained with other zinc ethylene-bis-dithiocarbamates (e.g., Dithane Z-78, Dithane D-14 + zinc sulfate) at equivalent concentration of active ingredient.

SUMMARY

Ascochyta ray blight of chrysanthemum has remained an important disease in North Carolina for more than 40 years without much spread to other areas. It occurs also in South Carolina, Mississippi, Florida, and Maryland. The fungus occurs in California. Sudden and severe commercial loss results from flower infection; stem and leaf invasion produces little injury.

Pycnidia are produced on petals, stems, or leaves under conditions of variable moisture and temperature. Conidia exude in gelatinous masses and are disseminated by water; at laboratory temperatures they germinate in 3½ to 4 hr. and infect petals in slightly more than 6 hr. Once the pathogen is established in the tissue it will continue growth and will cause the flower to decay under conditions of relative dryness. Infection and subsequent growth of the fungus in petals will occur at 6°–30° C., is highly favored at 20°–26° C., and is optimum at 24° C. Pycnidia form in petals within 13 days after infection. A very high biotic potential is mantained by the pathogen during the growing season.

Perithecia form quickly and profusely on infected stems, flowers, or leaves under moist, but also very dry conditions. They mature in late summer and fall, and appear to supply much of the primary inoculum for infection of developing buds and flowers in the fall and winter.

The previously unreported perfect stage of *Ascochyta chrysanthemi* Stev. is here described under the name, *Mycosphaerella ligulicola* sp. nov.

The fungus overwinters as mycelia or pycnidia, or as developing perithecia. Because of its wide tolerance of temperature and moisture it would seem to be a potentially dangerous parasite over a much wider area than its present range.

Various cultural practices designed to reduce inoculum carryover or to keep the budding or flowering chrysanthemum plants dry may aid in control. A Parzate spray (¾ lb. per 100 gal., plus a good spreader) applied on a 7-day schedule gave good control of the disease under field conditions.

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LOCAL SUSCEPTIBILITY OF COTYLEDONS AND LEAVES OF CUCUMBER TO TOBACCO MOSAIC VIRUS[1]

H. H. McKinney[2] and Robert W. Fulton[3]

Cucumber (*Cucumis sativus* L.) has long been regarded as being immune from all types of infection by the virus of tobacco mosaic, but during the past four years many tests have shown that this plant and several related species are locally susceptible to this virus, to a known mutant of the virus, and to several viruses thought to be related to the tobacco mosaic virus.

MATERIALS

At Beltsville, Maryland, the studies were conducted with the following seven virus isolates:

(1) Tobacco mosaic virus obtained from James Johnson in 1924.

(2) Mutant *BSY* (6), previously designated Type. A yellow mosaic virus (4), which was isolated from the tobacco mosaic virus listed above.

(3) Tobacco mosaic virus obtained from H. A. Allard.

(4) Aucuba yellow mosaic virus of tobacco from John Caldwell, Essex, England.

(5) A light green mosaic virus which was associated with the above aucuba yellow mosaic virus.

(6) A virus isolated from *Plantago major* L. (7). This virus induces symptoms in some individuals of *Plantago Rugelii* Dene., and it is very similar to, if not identical with, virus isolated from a few plants of *P. Rugelii* found near pathways and roadsides on the Station grounds at Beltsville, Maryland, and in Arlington, Virginia.

(7) Mild dark-green mosaic virus from *Nicotiana glauca* R. Grah. collected in the Canary Islands (3).

The tobacco mosaic virus from James Johnson has been used continuously by one of the authors (McKinney) since 1924, and it has been regarded as the type virus of tobacco mosaic in all of this author's studies. The mosaic mottling induced by this virus in tobacco has been illustrated in several publications (4, Fig. 1, B; 5, frontispiece B). The tobacco mosaic virus from H. A. Allard had been stored by him for 15 years previous to 1931, when a portion of it was used in the mutation studies (4). From 1931 to 1949 the

portion of the virus used in the present studies had been stored in air-dried tobacco leaf tissue at room temperature.

Beans (*Phaseolus vulgaris* L.) of the variety Scotia, *Datura stramonium* L., and the hybrid *Nicotiana tabacum* × *N. glutinosa* were used for assaying virus in the cucurbit tissues.

At Madison, Wisconsin, the studies were conducted with 8 strains of tobacco mosaic virus obtained from James Johnson (2), with the masked strain of tobacco mosaic virus isolated by Holmes (1), and with an undescribed virus isolate.

The undescribed virus was isolated from field-grown tobacco plants that had been severely stunted and had numerous small necrotic spots on slightly mottled leaves. When inoculated to the hybrid test plant, local lesions typical of tobacco mosaic virus were produced. The thermal inactivation point and resistance to drying were typical of tobacco mosaic virus, and in serological tests the virus was precipitated by antiserum to common tobacco-mosaic virus. The virus produced local lesions on healthy leaves of *Nicotiana sylvestris* Speg. and Comes, but no lesions were produced on leaves of this plant which had been inoculated 3 days before with common tobacco-mosaic virus. It was concluded that the new isolate is a strain of tobacco-mosaic virus. For convenience it is designated here as the KC strain.

METHODS

Inoculations were made by the wiping method, with and without the use of 600-mesh carborundum dust. In most of the tests the cucurbit cotyledons or leaves were inoculated with fresh virus extracts obtained from the leaves of growing tobacco plants. However, in some of the tests conducted at Beltsville the inoculations were made with virus that had been stored for long periods in air-dry leaf tissue at room temperatures. All plants were grown in small clay pots in greenhouses, or in culture chambers. At Beltsville, two methods of temperature management were followed: (1) 34° C. following inoculation and through the first night, followed by greenhouse temperature as near to 24° C. as possible to the end of the test; (2) as near 24° C. as possible, throughout the test. In bright warm weather the greenhouse temperatures were kept as low as possible by shade and ventilation. At Madison most of the cucumber tests were conducted in a greenhouse with the temperature controlled near 27° C., but some tests were conducted at higher temperatures in a culture chamber.

In the tests at Beltsville the multiplication of virus in the cucurbit cotyledons and leaves was determined by direct assay of these tissues at suitable times after inoculation. Tissues were diluted 3 to 5 times in distilled water. Some virus will remain on a wiped surface, even when rinsed under the stream of water from a faucet. To reduce error from this source, the inoculated cotyledons and leaves were soaked for 15 minutes in a M/0.1 solution of trisodium orthophosphate at room temperature. In some tests a

M/0.2 solution was used. The tissues were then rinsed thoroughly in virus-free water before being pulped and extracted for assay. Tests with virus-impregnated soft filter paper which was thoroughly dried and then soaked in solutions of trisodium orthophosphate leave no doubt as to the effectiveness of this chemical for killing residual virus on inoculated cotyledons and leaves. Never have more than the slightest traces of virus survived in the filter paper tests, and usually none survives.

In making the assays the lesion counts were calculated on a unit area basis. The areas of the wiped leaves of bean seedlings and Datura seedlings were determined within rather close limits by matching with paper patterns, the areas of which had been predetermined.

In the studies at Madison the multiplication of virus in the cucurbit cotyledons and leaves was determined by serial transfer inoculations from cucumber to cucumber, and by direct assay of the inoculated cotyledons on the hybrid test plant (*Nicotiana tabacum × N. glutinosa*). The lesion counts on this hybrid are given in terms of the total or average per leaf. It should be kept in mind that the area of one of these leaves is much greater than the area of a leaf of a bean seedling or of *Datura stramonium*.

RESULTS OF TESTS WITH CUCUMBER AND RELATED SPECIES

Symptoms Induced on Cucumber

Local lesions usually appeared within 4 to 7 days when cucumber cotyledons were inoculated with tobacco mosaic virus. Fresh virus from young tobacco plants, diluted 3 to 5 times in water, has induced 50 to 100 local lesions per cotyledon on Early White Spine cucumber. The lesions on cucumber are illustrated in figure 1. The yellow mosaic virus mutant *BSY*, the mild dark-green mosaic virus, aucuba mosaic virus, and the KC strain induced especially distinct lesions on cucumber cotyledons. The virus from *Plantago major* induced less distinct lesions. In several of the tests at both Beltsville and Madison lesions failed to appear on part or all of the cotyledons that had been inoculated with some of the viruses used. Assays showed that when the inoculum contained a sufficient amount of virus there was an appreciable amount of virus in these lesion-free cotyledons.

At Beltsville the cucumber varieties Early White Spine, National Pickling, Boston Pickling, Improved Long Green, and Early Cluster have shown nearly the same reaction characteristics. At Madison the KC strain produced similar symptoms on all of 17 varieties of cucumber.

Increase of Virus in Cucumber

The extent to which virus increase occurs in cucumber was demonstrated with dilutions of 10, 100, 1000, and 10,000 times in distilled water of an extract from cucumber cotyledons that had been inoculated 16 days previously with the Johnson virus. Any residual virus on the cotyledons was inactivated before processing. Each dilution sample was wiped on the pri-

mary leaves of 10 bean seedlings. Local lesion counts per cm² of leaf were 14.0, 4.5, 0.6, and 0.09, respectively, for the above four dilutions.

The Johnson virus heated for 10 min. at 87° C. infected one tobacco plant out of the 5 plants inoculated. Virus from this plant was used to inoculate the cotyledons and first leaf of 10 Early White Spine cucumber seedlings. The small chlorotic lesions that appeared were more numerous on the cotyle-
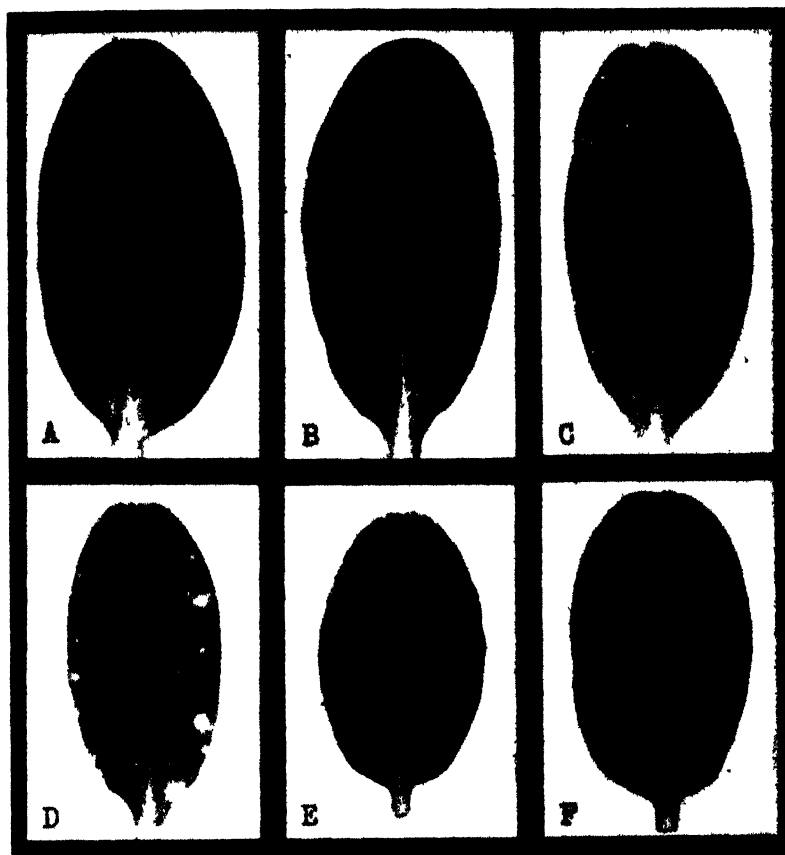
FIG. 1. Local lesions caused by strains of tobacco mosaic virus on the cotyledons of Boston Pickling (upper row) and Early White Spine (lower row) cucumber. A. Healthy control wiped with fresh juice from a healthy tobacco plant. B and D. Johnson's virus. C. KC virus. E. *BSY* virus. F. Virus from *Plantago major*.

dons than on the leaves. When the cotyledons were assayed on the leaves of *Datura stramonium*, local lesions were produced at the rate of 10 per cm.² of leaf. Assay data on seven virus isolates are shown in table 1. Variations in the lesion counts shown in the table are attributed chiefly to differences in the activity of the virus used to inoculate the cucumber cotyledons. The two lowest counts shown in the table are from virus that had been stored in dry tissue for long periods at room temperature, and used directly to inoculate the cotyledons.

TABLE 1.—*Assay data indicating an increase of virus in the cotyledons of cucumber inoculated with several collections of tobacco mosaic virus. Tissues were diluted 3 to 5 times in distilled water. Datura stramonium L. was used for the assays*

Virus used	Variety of cucumber inoculated	Virus in cucumber cotyledons (No. of lesions per cm^2 of test leaf)
Tobacco mosaic (James Johnson)	Early White Spine Boston Pickling	30.0 7.4
Tobacco yellow mosaic (Yellow mosaic mutant *BSY*)	Early White Spine Early Cluster	40.0 2.6
Tobacco mosaic (H. A. Allard)	Early White Spine	30.0
Tobacco aucuba (yellow) mosaic	do	10.0
Tobacco mosaic associated with aucuba mosaic	do	11.0
Mild dark-green mosaic from *Nicotiana glauca*, Canary Islands	do	30.0
Isolate from *Plantago major*	do	40.0

Data obtained at Madison, and presented in table 2, showed that several of the isolates of tobacco mosaic virus can be maintained through 5 to 10 serial passages on cucumber. The cucumber cotyledons from each transfer were assayed on the hybrid test plant. With some of the viruses (KC, isolates 1, 2, and 5, and Holmes' masked), cucumber cotyledons from the last transfer produced as many lesions as from the first. Other isolates could not be maintained on cucumber, perhaps partly because the original inoculum did not contain sufficient virus.

New leaves and stems of cucumber plants that had been inoculated previously by wiping the cotyledons and first leaf with the several viruses under

TABLE 2.—*Results of serial transfers at 6- to 8-day intervals of 10 isolates of tobacco mosaic virus in the cotyledons of cucumber*

Strain of virus used	Number of transfers from cucumber to cucumber	Average number of local lesions per hybrid test leaf inoculated with cucumber cotyledons from each transfer
KC	10[a]	83
1	6[a]	131
2	6[a]	114
3	3[b]	4
4	2	29
5	5[a]	41
6	4	54
7	1[b]	5
8	2	16
Holmes' masked	6[a]	40

[a] No attempts were made to carry these transfers further.
[b] Two additional attempts failed to maintain each of these viruses on cucumber.

study were assayed on many occasions. In no case was it possible to detect virus in these tissues, which indicated that the virus had not moved in from the inoculated sites. At Beltsville, one plant of cucumber, variety National Pickling, was allowed to grow for about 1 month after inoculation with the Johnson virus through the cotyledons and first leaf. At the end of this period the right half of each leaf from the second to the seventh inclusive was inoculated with the Johnson virus. A few small chlorotic lesions appeared on the leaves. Each inoculated half-leaf was assayed on the primary leaves of 5 bean seedlings. Local necrotic lesions were produced at the rate of 3.75, 0.65, 0.50, 1.67, 2.32, and 0.55 per cm², on test leaves 2 to 7, inclusive. The cucumber half-leaves that were not inoculated were bulked and assayed on the primary leaves of 5 bean seedlings. No local lesions or other signs of infection appeared.

In trials at Madison, cucumbers were inoculated with several of the virus isolates under study, and the plants were cultured at 35° C. and at 38° C. for 8 to 10 days. After an additional 14 days at 27° C. the leaves and cotyledons of the plants were assayed separately. No virus could be demonstrated in the noninoculated leaves, while the inoculated cotyledons produced from 30 to 100 lesions per leaf on the hybrid test plant.

Tests on Other Cucurbits

The cotyledons of watermelon (*Citrullus vulgaris* Schrad., var. Dixie Queen), squash (*Cucurbita pepo* L. var. White Scallop Bush), and gourds (*Cucurbita pepo*, mixed) were inoculated with a very active fresh extract of the Johnson virus. The lesions on watermelon were well-defined necrotic spots, a few of which penetrated to the underside of the cotyledons. The lesions on squash and gourds were faint gray specks that were very slow to appear. Assays on *Datura stramonium* after 10 days gave 2.4, 7.0, and 7.5 lesions per cm² of leaf, respectively, for the cotyledons of watermelon, squash, and gourd. In spite of the well-defined lesions on the cotyledons of watermelon, the assays have always shown low virus activity in this species.

The KC strain of virus was inoculated to cotyledons of watermelon (var. Stone Mountain), squash (var. Butternut), pumpkin (*Cucurbita pepo* L. var. Connecticut Field), and muskmelon (*Cucumis melo* L. var. Bender's Surprise). The lesions on watermelon were small but distinct orange to brown spots. On the other species the lesions were indistinct. Assays from inoculated cotyledons gave from 30 to 80 lesions per leaf of the hybrid test plant, except in the case of watermelon, from which virus could not be recovered.

CONCLUSIONS

It has been known for many years that the tobacco mosaic virus does not infect cucumber systemically, and apparently it had been assumed that this host was also immune from local infection. However, it appears from the results presented that the capacity for infecting cucumber locally is a rather

general property of this virus and its strains. Failure to observe this phenomenon in the past can be explained by the fact that the local lesions are not always striking on cucumber cotyledons, especially when the concentration of virus in the inoculum is not reasonably high.

It is of interest that the mild dark-green mosaic virus, which is regarded as being more distantly related to the tobacco mosaic virus than an ordinary mutant, also induces local infection in cucumber.

It appears that cucumber and certain other cucurbits have greater value than has been supposed for the identification of tobacco mosaic virus and some of its mutants and more distant relatives.

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INCIDENCE OF LIMA BEAN ROOT ROT IN SOILS TREATED WITH FUMIGANTS AND INSECTICIDES FOR CONTROL OF WIREWORMS[1]

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INTRODUCTION

Lima beans (*Phaseolus lunatus* L.) grown in the southern coastal counties of California are subject to root rot caused by *Fusarium solani* f. *phaseoli* (Burk.) Snyder and Hansen, *Pythium ultimum* Trow, *Rhizoctonia solani* Kühn, and *Thielaviopsis basicola* (Berk.) Ferraris, individually or collectively (13). The results of a several years' survey of areas producing Lima bean seed seemed to indicate that root rot is generally more prevalent in light soils than in heavy soils, and further, that these light soils usually contain relatively large populations of nematodes (*Heterodera marioni* (Cornu) Goodey) and of the sugar-beet wireworm (*Limonius californicus* Mann.). This suggested a possible relationship between soil fauna and root rot of Lima beans. With the advent of measures devised to control both nematodes and wireworms, an opportunity was afforded to check this hypothesis.

The Ventura County survey to determine the incidence of Lima bean root rot was conducted in field plots located on the Borchard and Bruns ranches near Oxnard, California. These plots were also used for testing the efficacy of various soil fumigants and insecticides for the control of the sugar-beet wireworm, and for two years (1946–1947) data were collected for the purpose of testing the validity of the contention that the soil fauna preying on Lima bean roots predisposes the host to infection by the several root-rotting organisms. Results are reported in the present paper.

MATERIALS AND METHODS

A dichloropropane-dichloropropylene mixture and an ethylene dibromide solution were tested as soil fumigants, and technical benzene hexachloride and technical dichlorodiphenyltrichloroethane (DDT) were tested as insecticides. The dichloropropane-dichloropropylene mixture contained approximately 33 per cent 1,2 dichloropropane, 63 per cent 1,3 dichloropropylene, and 4 per cent heavy trichlorides of propane. The ethylene dibromide solution contained 10 per cent ethylene dibromide by volume in a naphtha 200-base thinner. The technical benzene hexachloride con-

tained 12 per cent gamma isomer. The DDT and benzene hexachloride were diluted with pyrophyllite before application.

In each experiment the treatment and control were replicated four times in randomized blocks. Lima beans of the Ventura variety were planted in each field between May 3 and 10, at the rate of 90 lb. per acre. The wireworm population of the bean rows was determined by taking eight soil samples 4 in. wide, 4 in. deep, and 3 ft. long, at random, in the center 10 rows of each plot, between the fifth and seventh days after planting. Each sample was sifted through a 12-mesh screen to recover the wireworms present.

Soil fumigants were tested in ¼-acre plots of Yolo fine sandy loam soil in Borchard field A (Table 1). The dichloropropane-dichloropropylene mixture was applied at the rate of 18, 26, and 36 gal. per acre, and the ethylene dibromide solution at the rate of 22 gal. per acre. These materials were applied 6 to 7 in. deep, at 12-in. spacings, by means of a small, continuous-flow-type soil-fumigant applicator. At the time of treatment (April 10, 1946), the moisture content of the soil ranged from 6.4 per cent, by weight, at the 4-in. depth to 13.1 per cent at the 12-in. depth; soil temperatures ranged from 64° to 67° F. at the 6-in. depth and from 63° to 66° at the 12-in. depth.

The same soil fumigants were also tested in ¼-acre plots of Yolo very fine sandy loam in Bruns field A (Table 1). The dichloropropane-dichloropropylene mixture was applied at the rate of 22 gal. per acre, and the ethylene dibromide solution at the rate of 21 gal. per acre. These materials were applied 7 to 8 in. deep, at 12-in. spacings, by means of a commercial soil-fumigant applicator. At the time of treatment (April 8, 1947), the moisture content of the soil averaged 7.3, 9.1, 10.7, and 12 per cent, by weight, at the 4-, 8-, 12-, and 16-in. depths, respectively; soil temperatures ranged from 56° to 68° F. at the 4-in. depth and from 60° to 63° at the 12-in. depth.

Insecticides used in Borchard field B (Table 1) were applied as dusts on the soil surface of plots ⅝-acre in size. The materials, benzene hexachloride (4 lb. per acre) and DDT (20 lb. per acre), were incorporated with the soil by disking and cross-disking to a depth of 8 in. and then roll-harrowing. At the time of treatment (April 23, 1946), this Yolo fine sandy loam soil had a moisture content of 11.1, 14.4, 16.2, and 18.1 per cent, by weight, at the 4-, 8-, 12-, and 16-in. depths, respectively; and soil temperatures ranged from 63° to 65° F. at the 6-in. depth.

These same materials were also tested in Bruns field B (Table 1) in a randomized block experiment, with four replicated plots ¼-acre in size for each treatment. The benzene hexachloride was applied at the rate of 4 lb. per acre, and the DDT at 10 lb. per acre. The test area had been planted to a covercrop of sweet clover, and the dust treatments were applied on the covercrop before it was plowed under. At the time of treatment (March 20, 1947), the Yolo very fine sandy loam soil in these plots was

rather wet, owing to a previous irrigation. Soil moisture averaged 11.5, 13.0, 13.4, and 13.5 per cent, by weight, at the 4-, 8-, 12-, and 16-in. depths, respectively; and soil temperatures averaged 70°, 66°, and 65° F. at the 4-, 8-, and 12-in. depths, respectively.

Samples consisting of 10 bean plants from each of four replicate plots were collected about 6 weeks after seeding. A root-rot injury rating of the several samples was made, utilizing five arbitrary classes from 0 to 4, which represent approximately 0, 25, 50, 75, and 100 per cent injury of the hypocotyl surface. This rating was transformed into an injury index in which the index is the sum of the products of each class value and the number of individuals within that class, divided by the total number of individuals in the sample. A portion of each rated hypocotyl was removed and plated on cornmeal agar. The cornmeal agar plates were examined after sufficient time had elapsed to permit all fungus growth to become evident, and the total number of fungus colonies was then recorded.

RESULTS

It may be observed from the data presented (Table 1) that the effect of all treatments was to reduce the value of the injury index.

The fungi isolated (Table 1) comprised principally those species concerned with the root-rot complex, but there were also occasional isolates of *Fusarium oxysporum* Schl., *F. roseum* (Lk.) Snyder and Hansen, *Rhizopus* sp., and *Trichoderma lignorum* (Tode) Harz.

Wireworm control (Table 1) was effected by all treatments, with greatest population decrease with soil applications of ethylene dibromide solution. No data were secured for nematode populations because of the lack of a suitable technique permitting population estimates upon immature plant samples.

There is a direct relationship between the injury index and the number of fungi isolated from the bean plants sampled in all plots. This relationship is more conclusively illustrated by the ratio of the injury index to the number of fungi isolated (Table 1), and notable uniformity of correlation may be noted within the three areas sampled.

There is likewise a direct relationship between the number of wireworms per foot of bean row and the injury index, as illustrated by the calculated ratios (Table 1). A comparison of the two classes of ratios given reveals immediately that there is a proportionately greater reduction in wireworm population with treatment than there is corresponding decrease in injury index.

EFFICACY OF MATERIALS AS FUNGICIDES

Dichloropropane-dichloropropylene mixture and ethylene dibromide solution have only recently been used as soil fumigants; this limits the accumulation of much pertinent literature.

TABLE 1.—*The incidence of Lima bean root rot in field plots treated for the control of the sugar beet wireworm*

Treatment, plot location, and material used	Amount per acre	Root-rot injury index	Number of fungi isolated	Number of wireworms per foot of bean row[a]	Ratio of	
					Injury index to number of fungi	Number of wireworms to injury index
Soil fumigant						
Borchard field A:						
Dichloropropane-dichloropropylene mixture	18 gal.	2.7	49	0.3	0.06	0.11
do	26 gal.	2.2[b]	36	0.2	0.06	0.09
do	36 gal.	1.8[b]	28	0.1	0.06	0.06
Ethylene dibromide solution	22 gal.	2.8	43	0.0	0.06	0.00
Control (no treatment)		3.6	63	5.5	0.06	1.53
Bruns field A:						
Dichloropropane-dichloropropylene mixture	22 gal.	1.6	87	0.18	0.02	0.11
Ethylene dibromide solution	21 gal.	1.7	63	0.02	0.03	0.01
Control (no treatment)		2.3	89	1.03	0.03	0.45
Insecticidal dust						
Borchard field B:						
Benzene hexachloride	4 lb.	2.4	56	0.2	0.04	0.08
DDT	20 lb.	2.5	47	0.7	0.05	0.28
Control (no treatment)		2.7	67	3.1	0.04	1.15
Bruns field B:						
Benzene hexachloride	4 lb.	1.4	64	0.2	0.02	0.14
DDT	10 lb.	1.4	71	0.3	0.02	0.21
Control (no treatment)		2.2	87	3.8	0.02	1.73

[a] A difference of 1.1, 0.8, 0.2, and 0.8 wireworms per foot of soil (4 × 4 × 12 in.) required for significance at 5 per cent level in Borchard fields A and B and Bruns fields A and B, respectively.

[b] Significantly different from the control at the 5 per cent level, according to the ''t'' test for group comparison.

Carter (2) first reported the use of dichloropropane-dichloropropylene mixture as a nematocide in 1943. Application of 15 gal. (150 lb.) of this mixture per acre corrected pineapple plant failure, which Carter states is induced by "at least *Anomala* beetle larvae (*A. orientalis*), nematodes and pythiaceous fungi." The pythiaceous fungi presumably include *Phytophthora cinnamomi* Rands, *P. parasitica* Dast., and *Pythium* spp. (9).

Parris (12) reports dichloropropane-dichloropropylene mixture to be an efficient nematocide but a poor fungicide, at dosages of 100 gal. per acre, for the control of damping-off of pea and spinach in soil infested with *Rhizoctonia* cf. *solani* Kühn and *Fusarium* cf. *martii* A. and W., and tomato wilt caused by *F. oxysporum* f. *lycopersici* (Sacc.) Snyder and Hansen. Dosages of 55 gal. per acre did not reduce post-emergence loss of beans caused by *Pythium aphanidermatum* (Edson) Fitzpatrick. Newhall (10) confirms the finding that dichloropropane-dichloropropylene mixture is effective as a nematocide, and adds that "it has rather limited fungicidal and herbicidal properties."

Stark and Lear (14) report favorably upon the nematocidal properties of both dichloropropane-dichloropropylene mixture and ethylene dibromide solution, and add that dichloropropane-dichloropropylene mixture has fungicidal qualities at dosages higher than required for nematode control, but that ethylene dibromide solution was a relatively poor fungicide. The addition of 0.50 ml. of dichloropropane-dichloropropylene mixture to a 1-gal. glazed crock containing soil infested with *Fusarium* sp. permitted significantly greater emergence of pea seedlings than untreated soil. If we may assume that the 0.50-ml. dosage is received by a gallon of soil, such a dosage may be converted to gallons per acre by calculating the number of gallons of soil in an acre-foot and relating the charge applied. The milliliter dosages given by Stark and Lear (14) may then be multiplied by the factor 85.5 and expressed as gallons per acre. A 0.50-ml. dosage is therefore equivalent to 42.7 gal. per acre. This dosage of ethylene dibromide solution caused injury to pea seeds sown 8 to 14 days after treatment. A dosage of 0.25 ml., or 21.4 gal. per acre, did not cause pea-seed injury and failed to reduce significantly the damping-off of peas.

Zentmyer and Klotz (18) report that *Phytophthora cinnamomi* and *P. citrophthora* (Sm. and Sm.) Leonian are killed in soil by a mixture of 50 per cent 1,2 dichloropropane and 50 per cent 1,3 dichloropropylene, at a dosage of 0.15 ml. per qt. of soil, or about 51 gal. per acre; and by ethylene dibromide solution at a dosage of 0.27 ml. per qt. of soil, or approximately 92 gal. per acre. Using the above components separately, they determined that 1,2 dichloropropane was nontoxic to the fungi in soil at dosages of about 20, 51, and 92 gal. per acre; and that 1,3 dichloropropylene was nontoxic at about 20 gal. per acre, but was toxic at dosages of approximately 51 and 92 gal. per acre.

McClellan, Christie, and Horn (8) recently reported that under the con-

ditions of their experiments dichloropropane-dichloropropylene mixture was relatively ineffective, and ethylene dibromide solution had no effect, against *Fusarium oxysporum* f. *callistephi* (Beach) Snyder and Hansen, but that ethylene dibromide did reduce the viability of sclerotia of *Sclerotium rolfsii* Sacc. The fumigants were applied at the rate of 2.5 ml. to 46.5 lb. of soil in containers having a calculated capacity of about 7 gal. The description of methods does not permit ready determination of dosage, but the present writers estimate the 2.5-ml. charge to be equivalent to 23 to 30 gal. per acre.

Despite the large volume of literature published on DDT, there is a paucity of information concerning its value as a fungicide. Granovsky (3, 4) reported that 5 per cent DDT dust formulations had considerable fungicidal value for the control of late blight of potatoes, caused by *Phytophthora infestans* (Mont.) de Bary. Norris (11) published data demonstrating conclusively that 1 per cent DDT in culture media was nonfungicidal to *Ascochyta imperfecta* Pk., *Colletotrichum trifolii* Bain, *Ophiobolus graminis* Sacc., *Pleospora herbarum* (Pers.) Rab., and *Pseudoplea trifolii* (Rostr.) Petr. Norris also demonstrated that pea seed dusted with 50 per cent DDT and planted in infested soil gave 0 to 1 per cent emergence, whereas pea seed dusted with Spergon (tetrachloroparabenzoquinone) and sown in the same soil gave 86.5 per cent emergence. He further reports that undiluted DDT did not prevent development of wheat flag smut, caused by *Urocystis tritici* Körn. Heuberger and Wolfenbarger (6) ascertained that 0.75 lb DDT per 100 gal. of water, alone and in combination with copper oxychloride or zinc dimethyldithiocarbamate, had no effect upon the control of early blight of potato, caused by *Alternaria solani* (E. and M.) Jones and Grout. Abbott (1), working with the slime mold *Physarella oblonga* Morgan, showed that there was no reduction in growth when the amount of DDT added to the medium was varied from 1.6 to 105.0 mg. per ml. Surface applications of DDT at the rate of 40 mg. per sq. cm. retarded the growth rate but not the ultimate amount of growth; reduction of growth rate was attributed to physical change of the surface of the medium. Horsfall (7) indicates that DDT is not fungicidal in conjunction with studies directed toward developing new fungicides. Timonin (15) demonstrated that 5 to 20 mg. of DDT per 100 gm. of culture medium was nontoxic to *Saccharomyces ellipsoideus* R., but that alcohol production was decreased and the rate of fermentation delayed for the initial 5 days. Similar results were secured with grapes sprayed with DDT. Wilson and Choudhri (16) report that DDT had no effect upon the growth of pure cultures of *Rhizobium* spp. from sundry leguminous hosts, and did not inhibit growth of *Aspergillus niger* v. Tiegh., *A. oryzae* (Ahl.) Cohn, *Penicillium camemberti* Thom, *P. expansum* Lk., *P. italicum* Weh., *P. roqueforti* Thom, *Chlorella* sp., 6 unidentified species of fungi, 10 species of bacteria, 5 species of algae, and 3 species of Actinomycetes. Heuberger and Stearns (5) state that "DDT has little or no fungicidal value."

Wilson and Choudhri (17) report that the application of 20 per cent crude benzene hexachloride to a number of heterotrophic soil organisms did not affect their ultimate growth, but that the growth of three species of algae was inhibited. The growth of pure cultures of *Rhizoctonia solani* was inhibited by the presence of pure delta isomer; no information was given for the effect of gamma isomer on this fungus.

DISCUSSION

The data (Table 1) show that there is a direct relationship between treatments and the amount of Lima bean root rot; they also show that mature wireworm populations were reduced by the treatments, but that the rate of reduction of wireworm populations was greater than the rate of reduction of disease incidence. This suggests not only that there is a relationship between wireworm populations and the incidence of Lima bean root rot, but also that treatment directly affects the incidence of the disease. Since there is a more rapid reduction of wireworms than of injury indices, it is assumed that wireworms play only a minor role in predisposing Lima beans to root rot. This is illustrated by the data, which show that the injury indices decrease with increases in dosages of dichloropropane-dichloropropylene mixture, whereas the wireworm populations are not so effectively reduced by applications of this mixture as by applications of ethylene dibromide solution; the fungicidal efficacy of dichloropropane-dichloropropylene mixture is thus demonstrated.

Treatment with ethylene dibromide solution reduced the injury index below that of the untreated plot. This reduction may have been due almost entirely to the elimination of the predisposing action of wireworm populations rather than to any fungicidal activity of the material. Similarly, benzene hexachloride and DDT treatments reduced the injury index below that of untreated areas, although less than did dichloropropane-dichloropropylene mixture; this again suggests that the reduction of injury indices was due more to the elimination of the predisposing effect of wireworms than to any fungicidal action.

Except for the reports of Granovsky (3, 4) and Parris (12), the preceding review of the relative fungicidal efficacy of the materials used would apparently support the contention that only the dichloropropane-dichloropropylene mixture has fungicidal properties. Granovsky fails to present sufficient data to permit ready interpretation of his conclusion, and until additional information is supplied the writers feel obliged to consider DDT as having little or no fungicidal value. Parris demonstrates conclusively that, at dosages employed, dichloropropane-dichloropropylene mixture was not an effective fungus eradicant, although at maximum dosages there was apparently a decrease in fungus populations. Since the results and interpretations presented here are concerned primarily with changes in numbers of fungi, and not their eradication, the findings of Parris are not alien to the problem discussed.

Root rot of Lima beans may operate independently of wireworm populations, but it is evident that there is a relationship between the soil fauna and the incidence of root rot, as hypothesized, and that wireworms may predispose the beans to root rot. Although no data were secured on nematode populations, it is interesting to note that applications of ethylene dibromide solution, which is reportedly an efficient nematocide, did not substantially reduce the amount of root rot in any trial, despite the virtual elimination of wireworm populations. This suggests that nematodes play little if any part in predisposing Lima beans to root rot.

SUMMARY

Root rot of Lima beans (*Phaseolus lunatus* L.) is a complex involving the fungi *Fusarium solani* f. *phaseoli* (Burk.) Snyder and Hansen, *Pythium ultimum* Trow, *Rhizoctonia solani* Kühn, and *Thielaviopsis basicola* (Berk.) Ferraris.

The injury index is directly related to the number of fungi present on the subterranean portion of infected Lima bean plants.

Applications of the fumigants dichloropropane-dichloropropylene mixture and ethylene dibromide solution, and of the insecticides benzene hexachloride and DDT, to soil supporting Lima beans reduced the injury index over that for Lima beans grown on untreated soil.

A relationship exists between the number of wireworms and the injury index, although this relationship is not so pronounced as that between the injury index and the number of fungi. In general, the number of wireworms decreases more rapidly with treatment than does the injury index.

Reduction in disease incidence seems to be associated more closely with liquid applications, especially with dichloropropane-dichloropropylene mixture, than with dust applications. This suggests a fungicidal effect due to dichloropropane-dichloropropylene mixture.

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THE ACTION OF ANTIBACTERIAL SUBSTANCES ON THE GROWTH OF PHYTOMONAS TUMEFACIENS AND OF CROWN GALL TUMOR TISSUES[1]

R. S. DE ROPP

Action of a crude preparation of penicillin on the crown gall organism and on galls produced by this organism was described by Brown and Boyle (1), who found that the crude metabolic liquid from a culture of *Penicillium notatum* inhibited the growth of *Phytomonas tumefaciens in vitro*. When introduced into crown galls on *Bryophyllum* by means of needle puncture, it caused the recession of these galls. Subsequently, Brown (2) reported that both penicillin and streptomycin were capable of destroying gall cells without injuring normal cells, the antibiotic substances causing destruction of the nuclei of the gall cells. "Cures of crown gall" were also reported by Hampton (3) in various plants which had been treated with penicillin and streptomycin, both the commercial and locally produced crude preparations.

Evaluation of this work is made difficult by the fact that impure preparations of these antibiotic substances appear to have been used. Crude preparations of these substances contain many compounds which may exert some action on plant growth. Also the findings of Brown and Boyle regarding the sensitivity of *Ph. tumefaciens* to penicillin appear to conflict with those of Waksman *et al.* (6), who state that, compared with Gram positive bacteria, *Ph. tumefaciens* must be considered as "fairly resistant" to penicillin. Experiments were therefore undertaken to explore this subject further, using the purest available preparations of antibiotic substances and employing tissue culture methods for growing both normal and tumor tissue.

MATERIALS AND METHODS

Three categories of experiments were performed. First, different strains of *Ph. tumefaciens* were treated in broth culture with different concentrations of antibacterial substances to determine what concentration of these substances would inhibit bacterial growth. Second, bacteria-free crown gall tissues of *Helianthus annuus* and *Vinca rosea* were cultured on a nutrient medium containing antibiotic substances and their growth compared with that made on nutrient agar lacking these substances. Normal tissues from these plants were similarly tested. Third, segments of sterile carrot tissue were inoculated with a virulent strain of *Ph. tumefaciens* and subsequently treated with antibiotic substances to determine whether these compounds had the capacity to inhibit tumor formation.

822

The antibacterial substances used in this work were obtained from the following sources: streptomycin calcium chloride complex from Commercial Solvents Corporation; crystalline potassium penicillin G and streptomycin from R. T. Major of Merck and Co.; chloromycetin from Parke, Davis and Co.; aspergillic acid from G. Rake of the Squibb Institute for Medical Research; patulin from H. Raistrick; tolu-p-quinone, recrystallized material from Eastman Kodak Co.; A 377 hydrochloride (aureomycin) from Lederle Laboratories.

The strains of tumor tissue used were K1 from an induced tumor on sunflower and V 60 isolated by White (7) from *Vinca rosea*. The mode of obtaining normal tissue of *Vinca rosea* and *Helianthus annuus* has been described previously (4). The carrot tissue was removed from washed carrots by means of a sterile cork borer of 1 cm. diameter. Cylinders of tissue thus removed were cut into 6-mm. lengths and cultured on sucrose agar as employed for the culture of tumor tissues. Suspensions of *Ph. tumefaciens* having approximately known viable counts were applied to the cut surfaces of these tissue fragments, which were subsequently treated with the antibiotic substances to be tested.

THE ACTION OF ANTIBACTERIAL SUBSTANCES ON THE GROWTH OF PHYTOMONAS TUMEFACIENS IN VITRO

This portion of the research was carried out by Dr. Annette Hervey using the method of serial dilution described in a previous publication (5). Five strains of *Ph. tumefaciens* having different cultural characteristics and different degrees of virulence were tested against seven antibacterial substances. The strains of bacteria were cultured 48 hr. in broth, diluted 1:1000, distributed in 1-ml. amounts among 10 tubes to the first of which 1 ml. of the antibiotic to be tested was added. By serial transfer of 1 ml. from tube to tube, a series of dilutions of the antibacterial substance was obtained. The tubes were incubated at 25° C. and readings were taken after 1, 2, and 3 days. The end point was taken as the last tube showing complete inhibition of bacterial growth. The concentrations of antibacterial substances which gave complete inhibition are shown in table 1.

Among some of the strains tested, big differences in sensitivity to the various antibacterial substances were observed. These differences were not correlated with the tumefacient power of the strain. The strains BP and B6, both of which were highly tumefacient, differed in their sensitivity to almost all the substances tested. Streptomycin, though active against both virulent strains, was relatively inactive against the semi-virulent strain, A6. Chloromycetin, though highly active against both the virulent strain BP and the avirulent strain SP, had little action on the other strains. Penicillin G, though it inhibited the growth of strain BP at a concentration of 4 γ per liter, was completely inactive against the other strains. The highest overall activity was shown by aureomycin. Even to this substance strain

A6 was one hundred times less sensitive than strain SP. It is not possible to determine, on the basis of present data, what factors were responsible for these great differences in strain sensitivity.

TABLE 1.—*Lowest concentrations (γ per ml.) of various antibacterial substances which inhibited growth of Phytomonas tumefaciens in broth (24-hr. readings)*

Antibacterial substance	Strain of *Phytomonas tumefaciens*				
	BP	B6	B2	A6	SP
Penicillin G	4.0	500	1000	1000	31.0
Streptomycin	1.5	4.0	0.2	50.0	12.5
Streptothrycin	0.8 unit	25 units	0.8 unit	25 units	3.2 units
Chloromycetin	0.2	31.0	31.0	2.0	0.8
Patulin	2.0	31.0	15.6	7.8	8.0
A 377 hydrochloride (aureomycin)	0.03[a]	0.06	0.025	1.0	0.01[a]
Aspergillic acid	8.0	63.0	63.0	63.0	8.0
Tolu-p quinone	4.0	62.0	31.0	15.6	4.0

[a] (42 hr. readings)

THE GROWTH OF NORMAL AND BACTERIA-FREE TUMOR TISSUE IN THE PRESENCE OF PENICILLIN AND STREPTOMYCIN

Nutrient agar was prepared containing streptomycin, commercial penicillin, and penicillin G in concentrations of 5, 50, and 500 γ per ml. On this agar, fragments of tumor tissue of *Helianthus annuus* and *Vinca rosea* were placed.. The fresh weight of each of these fragments was obtained by

TABLE 2.—*Ratio of final to initial fresh weights of normal and tumor tissue of Helianthus and Vinca rosea after 4 weeks culture in the presence of antibiotic substances (means of 20 estimates)*

Antibiotic substance	Concentration γ per ml.	Type of tissue			
		Vinca normal	Vinca tumor	Helianthus normal	Helianthus tumor
Commercial penicillin	5	5.35	4.18	17.47	6.81
	50	4.01	3.60	11.36	5.40
	500	3.00	3.71	7.82	2.85
Penicillin G	5			1.77	6.70
	50		.	8.50	6.00
	500			9.21	3.21
Streptomycin	5	3.11	4.24	2.52	7.03
	50	2.50	3.27	1.71	4.29
	500	2.41	1.73	1.78	2.12
No antibiotic		3.41	5.20	1.87	6.80

weighing the culture tube before and after addition of the tissue fragment. Initial dry weights were estimated from separate samples. Tissue from the stems of normal plants was obtained as previously described. After 4

weeks at 25° C. in continuous light, the tissues were removed from the cul-
ture vessels and their individual fresh weights and pooled dry weights were
determined. Twenty replicates of every treatment were used in this experi-
ment, and the ratio of final to initial fresh weights is shown in table 2.
Dry weight increases were found to be proportional to fresh weight in-
creases and have therefore not been included.

Evidently the growth of tumor tissue of both kinds was not signifi-
cantly influenced by the presence of commercial penicillin in the medium
unless the concentration of this substance was of the order of 500 γ per
liter. This sample of penicillin contained an amount of indoleacetic acid
sufficient to cause root production in normal tissue both of sunflower and,
to a lesser extent, of Vinca. The very great weight increase of normal
sunflower tissue in the presence of this substance was due to this produc-
tion of roots. Penicillin G had some inhibiting action on sunflower tumor
tissue at a concentration of 500 γ per ml. It induced root formation at a
concentration of 50 and 500 γ per ml. in normal sunflower tissue. Possibly
this effect was also due to traces of indoleacetic acid. Streptomycin ex-
erted a more powerful inhibiting action on the growth of both types of
tumor tissue, being active in a concentration of 50 γ per ml. and still
further reducing growth at 500 γ per ml. Even at this concentration,
however, it did not inhibit the growth of the tumor tissue completely.
Streptomycin reduced the growth of normal tissue to a much smaller ex-
tent than it did that of tumor tissue.

THE ACTION OF STREPTOMYCIN AND PENICILLIN ON TUMORS ON ISOLATED FRAGMENTS OF CARROT TISSUE

Cylinders of sterile carrot tissue 10 mm. diam. and 6 mm. high were
placed on slopes of nutrient agar and inoculated on their upper sur-
faces with a suspension of *Ph. tumefaciens,* strain BP. The suspension
was prepared to have a standard opacity and diluted to give an approxi-
mately known number of organisms. Preliminary experiments showed
that as few as ten organisms applied to the freshly cut surface of carrot
tissue would suffice to give rise to tumors which appeared as slight green
protuberances about 6 days after the inoculation of the fragment. A de-
tailed description of the structure and mode of origin of these tumors will
be published elsewhere. In this series of experiments attention was fo-
cused on the effect of applications of antibiotic substances on the develop-
ment and subsequent growth of these tumors.

In the first experiments of this series both the concentrations of anti-
biotic substances and the number of organisms in the inoculum were varied.
The disks of carrot tissue were inoculated as soon as they had been placed
on nutrient agar, the inoculum being evenly spread over the upper surface
of the stem fragment. One day after its inoculation each fragment was
treated with 0.05 ml. of a solution of antibiotic substance which was also

applied to the upper surface of the fragment. After incubation for 2 weeks in continuous light at 25° C., the number of fragments bearing tumors on their upper surfaces were counted. The results of this experiment are shown in table 3.

Both streptomycin and penicillin G were capable of reducing tumor formation in carrot fragments inoculated with *Ph. tumefaciens*, but streptomycin was far more potent in this respect than was penicillin. Thus, when the inoculum contained 50 million organisms, penicillin had no effect on tumor formation even in a concentration of $5000\,\gamma$ per ml. Streptomycin at this concentration completely inhibited tumor formation, and at

TABLE 3.—*Effect of antibiotic substances on tumor formation on carrot fragments inoculated with suspensions of Phytomonas tumefaciens containing different numbers of organisms. Figures show number of fragments out of ten on which tumors were apparent at the end of 2 weeks*

No. of organisms in inoculum	Concentration of antibiotic substance (γ per ml.)				
	$5000\,\gamma$	$500\,\gamma$	$50\,\gamma$	$5\,\gamma$	$0\,\gamma$
Streptomycin					
50×10^6	0	3	10	10	10
50×10^4	1	4	10	10	10
50×10^2	2	5	10	10	10
50	0	3	6	10	10
0	0	0	0	0	0
Penicillin G					
50×10^6	10	10	10	10	10
50×10^4	9	10	10	10	10
50×10^2	4	10	10	10	10
50	2	6	10	10	10
0	0	0	0	0	0

a concentration of $500\,\gamma$ per ml. reduced the incidence of tumors from 10 out of 10 to 3 out of 10. This greater efficiency of streptomycin was apparent at all levels of the inoculum; in fact, it appears that the tumor-inhibiting action of streptomycin at the higher concentrations was about ten times more powerful than that of penicillin G.

It was observed that the tumor-inhibiting action of these antibiotic substances was limited to the upper surface of the disk to which they were applied. Even on disks treated with high concentrations of streptomycin, tumors occasionally appeared on the sides of the fragments to which the bacteria had gained access. At concentrations of $500\,\gamma$ and over, streptomycin appeared to be toxic to the carrot tissue and inhibited cell proliferation both on inoculated and on noninoculated tissue.

To determine whether streptomycin was capable of causing the regression of tumors once they had formed, a series of fragments of carrot tissue was prepared, inoculated with a suspension of *Ph. tumefaciens* containing

about 5000 organisms per ml. and treated with a solution of 500 γ per ml. streptomycin 1, 2, 6, and 8 days after inoculation. The disks were incubated for 4 weeks, at the end of which time the number of fragments with tumors in each group of ten tissue fragments was recorded (Table 4).

Evidently streptomycin was capable of inhibiting tumor formation only when it was applied during the first few days after the tissue had been inoculated. When the tumors were already formed, application of streptomycin brought about a reduction in their rate of growth but did not cause their regression. This finding suggests that the action of streptomycin may be on the bacteria rather than on the tumor tissue itself, though further work is needed to make clear the manner in which this antibiotic substance exerts its effect.

TABLE 4.—*Frequency of tumor formation on carrot fragments treated with 500 γ per ml. streptomycin at different intervals after inoculation with Phytomonas tumefaciens. (Figures show number of fragments out of 10 with visible tumors)*

Treatment		Number of fragments with tumors
Untreated		10
Streptomycin applied after 1 day		1
Do	2 days	0
Do	6 days	10
Do	8 days	10

SUMMARY

Six strains of *Phytomonas tumefaciens* were grown in broth culture in the presence of the following antibacterial substances: penicillin G, streptomycin, streptothrycin, chloromycetin, patulin, aureomycin, aspergillic acid, and tolu-p-quinone. The concentrations of these substances needed to inhibit growth of *Ph. tumefaciens* varied greatly among the different strains. Aureomycin, chloromycetin, streptomycin, and streptothrycin showed the highest degree of activity.

Normal and bacteria-free tumor tissue of *Helianthus annuus* and *Vinca rosca* were cultured *in vitro* on agar containing different concentrations of commercial penicillin, penicillin G, and streptomycin. At a concentration of 500 γ per ml. both penicillin preparations had an inhibiting action on the growth of tumor tissue. The growth of normal tissue was stimulated by these preparations chiefly owing to the fact that they both induced root production, an effect probably due to traces of indoleacetic acid. Streptomycin inhibited the growth of tumor tissue more actively than did penicillin, being effective in a concentration of 50 γ per ml.

Sterile slices of carrot tissue were inoculated with *Ph. tumefaciens* and treated one day later with penicillin G or streptomycin. Tumor formation was inhibited to some extent by both these substances, streptomycin

being more powerful in this respect than was penicillin. Once the tumors had been formed they were not caused to regress by streptomycin though their rate of growth was slowed down. It appeared that the effect of the streptomycin resulted from its action on the bacteria rather than from action on the tumor cells themselves.

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RESISTANCE TO EARLY BLIGHT AND SEPTORIA LEAF SPOT IN THE GENUS LYCOPERSICON[1]

SETH BARTON LOCKE[2]

INTRODUCTION

Differences in amount of defoliation frequently have been observed among commercial tomato varieties exposed under field conditions to infection by various leaf-spotting fungi. Yet a number of investigators (1, 3, 4) have failed to discover any appreciable differences in resistance to infection by certain of these pathogens when tomato varieties were artificially inoculated under conditions which were highly favorable to infection. The author's experience in this connection has been the same. Using a laboratory technique described in detail elsewhere (10), small but statistically significant differences in susceptibility to early blight (*Alternaria solani* (E. and M.) Jones and Grout) and Septoria leaf spot (*Septoria lycopersici* Speg.) were indicated among 15 common tomato varieties. However, strains of the same variety from different sources and even individual plants from the same seed lot sometimes reacted differently. In no case was a degree of resistance indicated which was sufficiently high to affect appreciably the amount of defoliation in the field. It is thus apparent that the observed differences in amount of defoliation of tomatoes in the field are not exclusively due to differences in resistance to infection by the common leaf-spotting fungi. Such factors as growth habit, bearing on disease escape, and fruit load, affecting predisposition of the suscept to infection, enter into the picture (5, 7).

In the absence, among improved tomato types, of a source of practical resistance to early blight and Septoria leaf spot, it seemed desirable to extend the search for such resistance to include the so-called "wild" forms of *Lycopersicon esculentum* Mill. and to other *Lycopersicon* species. In 1939 a lot of seed was obtained from the Division of Plant Exploration and Introduction, Bureau of Plant Industry, which comprised the remnants of a collection made by Dr. H. L. Blood in South America in 1938. The names employed here are those published by Muller (11). This collection included

69 numbers embracing five *Lycopersicon* species and several forms and varieties. For the most part, only 10 or 12 seeds of each number were supplied, and in some cases only one or two plants were obtained from these. This material was subjected to laboratory tests for reaction to early blight and Septoria leaf spot early in 1940 and again early in 1941 and to a field test during the summer of 1940. A preliminary report of the results of these tests was made in 1941 (9).

REACTION TO INOCULATION WITH ALTERNARIA SOLANI

As shown in table 1, significant differences in lesion diameters resulting from inoculation with *Alternaria solani* occurred among the accessions representing the same species in all instances. However, only two accessions were significantly more resistant than other strains in their respective species groups in both the 1940 and the 1941 tests. One was a strain of *Lycopersicon hirsutum* Humb. and Bonpl., P. I. No. 127827[1,4], which was significantly more resistant than five out of six and four out of seven other strains of the same species in the 1940 and 1941 tests respectively. This strain had the smallest mean lesion diameter of any of the strains tested. The other strain mentioned, P.I. No. 126953 of *L. pimpinellifolium* (Jusl.) Mill., was significantly more resistant than 6 out of 20 and one out of 20 other strains of the same species in the 1940 and 1941 tests respectively. Its mean lesion diameter for the two tests was approximately twice that obtained with the *L. hirsutum* strain, P.I. No. 127827.

On the basis of the combined data of the two tests, the order of increasing mean lesion diameters for species was *Lycopersicon hirsutum*, *L. glandulosum* C. H. Mul., *L. esculentum*, *L. pimpinellifolium*, and *L. peruvianum* (L.) Mill.

REACTION TO INOCULATION WITH SEPTORIA LYCOPERSICI

Statistically significant differences in mean lesion diameters resulting from inoculation with *Septoria lycopersici* were obtained in all of the species groups excepting that of *Lycopersicon peruvianum* in the 1940 test, and only in *L. pimpinellifolium* in the 1941 test. The strains of the last-named species which showed significantly smaller lesions were not the same in the two tests in any instance. In the *L. hirsutum* group the significant difference in the 1940 test occurred between one strain, P. I. No. 126444, which produced a large lesion, and the remaining six strains all of which produced small lesions and which were not significantly different from one another in this respect. The more susceptible strain was not included in the 1941 test. With the exception of P. I. No. 126444, the mean lesion diameters obtained with *L. hirsutum* strains were smaller than that obtained with any other accession tested.

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[1] P.I. No. refers to the accession numbers of the Division of Plant Exploration and Introduction.

[4] P.I. No. 127827 was represented in these tests by a vegetatively propagated clone derived from a single plant. This species failed to produce seeds under the conditions which prevailed during these studies.

TABLE 1.—*Reaction of South American Lycopersicon importations to infection with Alternaria solani and Septoria lycopersici*

Species	P.I. No.[a]	Mean lesion diameter[b]				Defoliation[c] S. lycopersici 1940
		A. solani		S. lycopersici		
		1940	1941	1940	1941	
		mm.	mm.	mm.	mm.	Per cent
L. hirsutum	126444	5.74		1.76		35.8
	126445	4.74	6.30	0.16	0.10	9.0
	126446	4.16	4.92	0.12	0.00	10.0
	126447	3.36	5.56	0.08	0.16	15.0
	126449	3.82	6.48	0.08	0.22	
	126936	5.32		0.16		30.0
	127826		7.32		0.40	13.0
	127827	1.40	3.72	0.04	0.00	13.0
f. glabratum	134417		6.62			18.0
	134418		7.30		0.22	12.5
Species mean		4.08	6.03	0.34	0.16	17.6
L.S.D.[d]	(19:1)	2.39	2.64	0.36	2.92	
	(99:1)	3.45		0.53		
L. glandulosum	126438	3.48		1.28		21.0
	126439	5 44		0.48		21.6
	126440	5.14		0.72	0.44	51.6
	126441			0.18		
	126443	5.28				37.5
	126448	3.38		0.32		13.0
Species mean		4.54		0.60	0.44	28.9
L.S.D.	(19:1)	2.02		0.74		
	(99:1)			1.08		
L. peruvianum	126926	3.56	7.20	1.18	1.28	
	126928	5.12	7.50	0.34	1.56	19.0
	126929		7.04		1.84	15.8
	126930	4.02	8.08	0.56	1.68	31.6
	126944	5.24	8.20	1.42	1.58	12.5
	126945	4.94	8.64	1.28	1.06	35.1
	126946	5.16	8.32	1.08	0.74	25.0
var. dentatum	126935		7.96		1.80	28.3
	127830			0.46		31 0
	127831			0.94		27.0
	127832	5.18	7.80	1.10	2.32	25.0
var. humifusum	127828		6.48		1.62	17.5
	127829	5.97	7.40	1.02	1.60	14.6
Species mean		4.90	7.67	0.94	1 55	23.5
L.S.D.	(19:1)	1.64	2.15	0.98	1.26	
	(99:1)					
L. esculentum	126451		7.14		1.06	33.3
	127815	4.94	7.34	0.54	1.88	21.6
	127816	5.26	6.44	0.62		38.3
var. cerasiforme	126417	5.54	5.78	0.70	1.04	31.6
	126418		5.80		1.98	46.6
	126419	5.52		1.40		46.6
	126425	5.08	6.32		0.80	21.0
	126426		6.90		1.32	20.0
	126942	3.68	5.78		1.56	30.8

TABLE 1. *(Continued)*

Species	P.I. No.[a]	Mean lesion diameter[b]				Defoliation[c] S. lycopersici 1940
		A. solani		S. lycopersici		
		1940	1941	1940	1941	
		mm.	mm.	mm.	mm.	Per cent
	126950		6.38	1.62	1.70	31.0
	127814	4.20		0.66		9.1
var. *pyriforme*	126452	4.60		0.20		27.5
	126955	6.42	5.46	1.12	1.58	39 1
Species mean		5.03	6.33	0.86	1.44	29 0
L.S D.	(19:1)	1.88	1.68	0.96	1.32	
	(99:1)	2.73		1.40		
L. *pimpinellifolium*	126432	4.96	7 66	0.92	1.68	50.8
	126433	5.34	6.82		1.78	32 0
	126436	6.10	6.10		1.86	22 0
	126437		6.58		2.26	45.0
	126925	5.84	8.38	1.14		15.8
	126927		7.18	1.58	2.10	35.0
	126931		6.94		1 28	47 5
	126932	4.56		0 62		43.3
	126933	4 54	7 16	1.14	1 56	48 3
	126934	5 08		1 28		39.1
	126937	4.47		1 80		36 2
	126938		6.70		2 64	40 0
	126939	4 96		0 74		26.0
	126940	5 48	7.80	0.78	1.52	45 0
	126941	4.82		0 68		16.0
	126943	4.82	7.42	1.52	1.50	18.7
	126947	5 76	7.54	2.54		45.8
	126948	3.76	7.56		2.80	43.3
	126950	4.52				
	126951	4.18	7 58	1.46	0.74	57.6
	126952	5 18	8.32		0.62	16.2
	126953	3.76	6.52	1.50	0 72	50.8
	126954		7 60		1.84	40.0
	127805	3.92	6.68	1.56	2.38	21.6
	127806	4.72		2.08		21 6
	127807	6 02	7.28	1.78	2.24	
	127833	5 52	6.70		2.38	25.0
Species mean		4 99	7.33	1.36	1.77	34.9
L.S.D.	(19:1)	1.67	1.81	1.20	1.29	
	(99:1)	2.40		1.74	1.88	

[a] Accession numbers of the Division of Plant Exploration and Introduction, Bureau of Plant Industry, U. S. D. A.

[b] Laboratory tests.

[c] Field test.

[d] Least difference required for significance.

In both tests the order of increasing mean lesion diameter for species was *Lycopersicon hirsutum, L. glandulosum, L. esculentum, L. peruvianum,* and *L. pimpinellifolium.* In both tests *L. hirsutum* was significantly more resistant than all of the other species excepting *L. glandulosum.* Significant differences occurred among the remaining species groups in both tests, but they were not consistent in the two tests.

Field plantings of most of the numbers included in the laboratory tests, made in 1940 at the main station farm near Fayetteville, Arkansas, afforded an opportunity for comparing the results of artificial inoculation with the amount of defoliation occurring under natural conditions. Single plots of six plants each were arranged at random in the planting. Where fewer than six seedlings of a strain were available, the strain was represented by a clone of vegetatively propagated individuals. This was the case with the *Lycopersicon hirsutum* strains excepting two belonging to the form *glabratum* C. H. Mul., most of the *L. glandulosum* strains, and several *L. peruvianum* strains.

Early blight appeared in planting early in the season, but failed to develop sufficiently to account for appreciable defoliation. Septoria leaf spot, however, became severe, and toward the end of the season all susceptible strains were badly defoliated. On September 10, the percentage of defoliation was estimated for each plant, working across the plots in order to minimize the personal factor. An average percentage of defoliation for each accession, based upon six independent observations, appears in the right-hand column of table 1.

No true replications of strains were provided in the field test, since the six plants of each strain were placed together in a single plot. Consequently, differences between individual strains are apt to be overshadowed by effects associated with location in the planting. However, legitimate comparisons between species can be made because the individual strains comprising them were scattered at random over the planting.

With the exception of *Lycopersicon glandulosum*, the field defoliation data parallel the laboratory data for resistance to Septoria leaf spot. *L. hirsutum* showed the least defoliation and *L. pimpinellifolium* the most. The three remaining species, *L. glandulosum*, *L. esculentum*, and *L. peruvianum*, showed only slightly less defoliation than *L. pimpinellifolium*. The apparent susceptibility of *L. glandulosum* in the field test, in contrast to its apparent resistance in the laboratory test, may be associated with its lack of vigor, which may have been a contributing factor to its defoliation in the field.

DISCUSSION

Before drawing conclusions from the data presented here, it seems appropriate to compare them with those obtained by other investigators working with the same or similar material. In 1938, Butler (6) reported briefly on the amount of defoliation occurring in 265 *Lycopersicon* importations under field conditions in Tennessee. *Septoria, Alternaria,* and *Cladosporium* were mentioned as contributing to defoliation, but the relative importance of each in this respect was not stated. In 1942, Alexander, Lincoln, and Wright (2) reported on field tests in Ohio and Indiana for resistance to early blight and Septoria leaf spot in 488 *Lycopersicon* importations.

Andrus and Reynard reported in 1942 on laboratory and field tests for resistance to early blight (3) and in 1945 on laboratory tests for resistance to Septoria leaf spot (4).

Butler found that four strains of *Lycopersicon hirsutum* were almost free from leaf spots in his test (6). The Ohio and Indiana field tests indicated a high degree of resistance in ten lines of *L. hirsutum* toward both Septoria leaf spot and early blight (2). Andrus and Reynard reported a high degree of resistance to Septoria leaf spot in several *L. esculentum* × *L. hirsutum* hybrids (4). On the basis of field observations they report that *L. hirsutum* is resistant to early blight (3). The results of the laboratory and field tests reported here are in agreement with those of other workers (2, 3, 6) insofar as reaction to Septoria leaf spot is concerned. No field test for reaction to early blight was obtained in Arkansas, but the author's laboratory tests indicated that only one accession of *L. hirsutum*, P. I. No. 127827, showed marked resistance to early blight. Considerable variation exists among individual plants of a strain represented by a single P. I. number. Consequently, the material tested by different investigators probably was not genetically identical. With the exception of P. I. No. 127827, it also is highly probable that resistance toward early blight observed under field conditions in strains of *L. hirsutum* is associated with the high degree of sterility shown by this species in North America. Extent of fruit load has been shown to affect susceptibility to defoliation diseases in tomatoes to a marked degree (5, 7). In the writer's laboratory tests where fruit load was not a factor, P. I. No. 127827 was resistant to early blight.

Alexander *et al.* (2) state that, from limited field observations, *Lycopersicon glandulosum* did not appear to possess any resistance to the diseases studied. However, they rate (in their table 3) one strain, P. I. No. 126448, of this species as resistant to Septoria leaf spot and early blight in their field tests. This particular accession showed some resistance to Septoria leaf spot and early blight in the writer's laboratory and field tests (Table 1). As pointed out earlier, *L. glandulosum* is not so vigorous as the other species. Consequently it may appear to be susceptible in field defoliation tests.

Strains of *Lycopersicon peruvianum* have been judged resistant to one or the other of the diseases in question by each of the workers testing them. However, there appears to be no agreement with regard to the particular accessions which are resistant. In the Indiana report, many lines are listed as heterozygous with respect to their reaction to the two diseases. This may be the explanation for a great deal of the confusion surrounding the reactions of this group.

In the case of *Lycopersicon peruvianum* var. *humifusum,* a different explanation is possible. Although the field tests in Arkansas, Ohio, and Indiana indicate resistance in strains of this variety, the writer's laboratory tests indicate that they are susceptible to both diseases. As observed in Arkansas, plants of this variety are extremely open in growth habit, pro-

ducing long, trailing vines with widely spaced leaves. This growth habit obviously is less favorable for infection than the dense type because it permits rapid drying of the foliage after rain- or dewfall. The writer is inclined to look upon the lack of defoliation of this variety in the field as disease escape rather than disease resistance. This view is supported by results obtained by Wright and Lincoln (12) who crossed a strain of this variety, P. I. No. 127829, with the tomato variety, Indiana Baltimore. They reported in 1940 that resistance was recessive in the F_1 generation. In 1941, however, Lincoln (8) reported that all of approximately 2,200 F_2 plants from this cross were susceptible to early blight and Septoria leaf spot. It thus appears that, as soon as the open habit of *L peruvianum* var. *humifusum* was lost, the apparent field resistance to defoliation diseases was lost also.

Butler (6) found one large-fruited strain of *Lycopersicon esculentum* (P. I. No. 128285) from Argentina, which showed only a small percentage of defoliation in the field. Alexander *et al.* (2) rated this accession as susceptible to Septoria leaf spot in their field tests. Andrus and Reynard (4) tested several other lines showing resistance to Septoria leaf spot which, they considered, were of hybrid origin, that is, outcrosses of *L. esculentum* with either the currant or cherry tomato types, and with *L. hirsutum*. One strain of *L. esculentum* var. *cerasiforme* (P. I. No. 127814) showed some resistance to defoliation in the field in Arkansas but rated susceptible in the writer's laboratory tests and as moderately resistant in the Ohio field test (2).

Alexander *et al.* (2) list 14 accessions of *Lycopersicon pimpinellifolium* as moderately resistant to Septoria leaf spot, as indicated by small lesion size. Andrus and Reynard (3) place 13 out of 14 accessions of this species in their more resistant classes with respect to early blight. They consider these as significantly tolerant to this disease. They noted outstanding resistance to defoliation in the field in progenies of accession P. I. No. 79532. This accession was not tested by the writer, but it is listed as susceptible to both early blight and Septoria leaf spot in the Indiana report (2). Of the 27 *L. pimpinellifolium* accessions tested by the writer only two showed resistance to Septoria leaf spot in both laboratory and field tests. One of these, P. I. No. 126925, was rated in the Indiana report (2) as moderately resistant. The other, P. I. No. 126952, was listed in the same report as susceptible to this disease.

It thus appears that considerable variability exists in the material tested under the same accession numbers by different investigators. However, there is general agreement with respect to the resistance of *Lycopersicon hirsutum* accessions to Septoria leaf spot. The writer believes that his tests indicate accession P. I. No. 127827 of this species as possessing the highest degree of resistance to both early blight and Septoria leaf spot found in any of the material tested. The probable value of this strain of *L. hirsutum* for breeding disease-resistant tomato varieties will be discussed in a subsequent publication.

SUMMARY

Small differences in susceptibility to early blight and Septoria leaf spot were found among 15 common tomato varieties by means of artificial inoculation. Sixty-nine *Lycopersicon* importations from South America were tested in the laboratory and field in search of a source of resistance for breeding purposes. Some degree of resistance was found in accessions belonging to each of the five species represented. The highest degree of resistance to either disease was found in strains of *L. hirsutum*, and one accession, P. I. No. 127827, was found to possess a high degree of resistance to both diseases.

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WISCONSIN PEA STREAK[1]

D . J . H A G E D O R N A N D J . C . W A L K E R

Linford (6) in 1929 reported that the streak disease of pea (*Pisum sativum* L.) was widespread in the United States, but no description of a causal agent was given until 1938 when Zaumeyer (13) discussed the relationship of pea streak to several strains of the alfalfa mosaic virus. He named the virus inciting this disease pea streak virus 1. The following year another pea streak virus was described by Chamberlain (1) in New Zealand.

In Wisconsin, pea streak was evidently not noticeable in 1924 because Jones and Linford (5) made no mention of it when they reported on the pea disease survey made that year. However, in the same State in 1942 Walker and Hare (10) found pea streak in about 60 per cent of 654 pea fields surveyed and in an occasional field the damage was severe. In a study of pea virus incidence in Wisconsin in 1948, pea streak was found to be widespread in all of the 12 important pea-growing counties surveyed.

A study of the nature of the pea streak disease, typical of that found in Wisconsin pea fields, was begun in 1946.

METHODS AND MATERIALS

For greenhouse studies plants were grown in compost soil in 6-in. earthenware pots at temperatures from 20° to 24° C. Plants of Perfected Wales, a very susceptible variety, were used for maintenance of stock cultures and most experiments. The virus was transmitted readily by rubbing young but fully expanded leaves, which had been sprinkled lightly with carborundum powder, with a pad made by folding cheesecloth around macerated diseased tissue. Determinations of physical properties were made in a manner similar to that described by Johnson and Grant (4), the exception being that aging tests were conducted at a temperature of 22° C. (72° F.) instead of at 80° – 90° F.

Virus-free pea aphids (*Illinoia pisi* (Kalt.)) were maintained on healthy broad beans (*Vicia faba* L.) or on peas in an aphid-tight wire mesh cage. Similar cages were used for insect detention during tests.

Six to 14 aphids were placed on each plant, and were confined to the plants by glass lamp chimneys. In most cases aphids were transferred by removal of the leaf on which they were feeding to the desired location.

When close study was to be made of feeding periods, aphids were moved by use of a camel's-hair brush.

EXPERIMENTAL RESULTS

Symptoms on Pea

Pea streak is a very conspicuous disease in the field. The most obvious

FIG. 1. Pea plant showing symptoms of Wisconsin pea streak as seen in the field. Note stem and petiole streaking, failure of pods to fill, dorsal suture discoloration, chlorosis and wrinkling of apical foliage.

symptom is necrosis of the stem and petioles (Fig. 1). Light brown to purple, oblong, necrotic lesions of various sizes are scattered along the surface of these plant parts. A single large lesion, formed by the coalescence

of several small ones, sometimes extends the length of several internodes. Many times the stem is girdled by these necrotic areas. Streaking is often present on the peduncle, and the dorsal suture of the pod sometimes shows a brownish discoloration. The nodes are also discolored. Irregularly

FIG. 2. Symptoms of Wisconsin pea streak in the greenhouse. Plant on left healthy; others diseased. Note progressive wilting and slight reduction in size of diseased plants.

shaped, light brown, slightly sunken, necrotic areas occur on both leaves and pods giving them a roughened appearance. If plants are attacked when young, the pods which do develop fail to fill normally. Terminal

plant parts are slightly malformed as a result of internode shortening and apical leaf wrinkling and curling. A mild general chlorosis of the leaves on the upper half of the plant is often observed but neither vein clearing nor mottle is characteristic. Moderate axillary bud growth is common. Some plants die prematurely.

Symptoms of pea streak were much less severe in the greenhouse than in the field. A progressive wilting of the plant (Fig. 2) was more characteristic in the greenhouse. The first noticeable symptom was a steel-gray discoloration of the stem. At this time the nodes above the inoculated leaves showed a slight browning. A few days later the inoculated leaves were dried but remained attached and their stipules were wilting. The first set of leaves and stipules above those inoculated was chlorotic and beginning to wilt. As wilting of the foliage progressed upward, the stipule became chlorotic and wilted before the leaf at the same node. In some cases, the uppermost leaves and stipules had become chlorotic 11 to 14 days after inoculation. At this time small, brown, necrotic streaks could sometimes be seen in the veins of leaves and stipules and on the stems. The browning at the nodes was conspicuous and the entire stem was grayish-tan. After 14 or 15 days, many plants had wilted completely and were dead. On others the stem streaks were from a few millimeters to several centimeters in length, even extending from one internode into another. The upper two sets of leaves and stipules were still turgid, but all other foliage had wilted. Stunting of infected plants was apparent occasionally. This symptom was caused, in part, by a slight reduction of internode length, but mainly by failure of the plant to continue its growth normally. A curling over of the apical growth sometimes occurred a few days before complete wilting and death. No vein clearing or mottle was observed.

Symptoms on Other Legumes

The Wisconsin pea streak virus incited vein clearing, and later mosaic symptoms on red clover (*Trifolium pratense* L.). Chlorosis along the main vein was conspicuous and small splotches of dark green tissue were dispersed irregularly over the mottled leaflets (Fig. 3, A). On alsike clover (*Trifolium hybridum* L.) vein clearing was the first symptom and later faint, small, oblong, chlorotic areas developed along the veins and between them, giving a somewhat mottled effect (Fig. 3, B). Infected leaves of this host were slightly wrinkled. Infected white sweet clover (*Melilotus officinalis* (L.) Lam.) displayed symptoms of mosaic—a few dark green splotches and chlorotic areas with definite margins scattered over the leaflet (Fig. 3, E). Symptoms on yellow sweet clover (*M. alba* Desr.) were vein clearing, mottle caused by interveinal chlorotic spots of various sizes and shapes, and a general loss of green color throughout the entire leaf (Fig. 3, D). On Ladino white clover (*Trifolium repens* L.) the only

symptom was a very slight leaf crinkle. Infected soybeans (*Soja max* (L.) Piper) showed very faint general chlorosis.
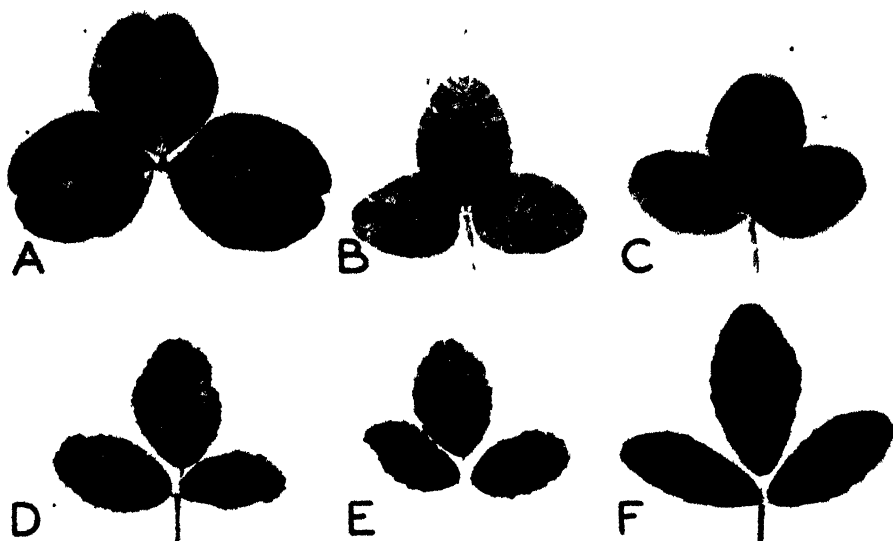
FIG. 3.   Symptoms of the Wisconsin pea streak virus on clover leaves. A, diseased red clover; B, diseased alsike clover; C, healthy alsike clover; D, diseased white sweet clover; E, diseased yellow sweet clover; F, healthy white sweet clover.

Effect of Temperature upon Disease Development

The effect of air temperature upon disease development on pea was studied at 16°, 20°, 24°, and 28° C. At 16° C. symptoms did not appear until 25 days after inoculation. Brown streaks on the stem were much more severe at this temperature than at higher temperatures and death from progressive wilting was delayed as long as 45 days after inoculation. When inoculated plants were grown at 20° C., initial disease symptoms could be seen as early as the seventh day and plants often were dead 10 days later. Symptoms at this temperature were similar to those previously described. Inoculated plants growing at 24° C. and 28° C. developed typical symptoms in seven days. Death occurred 15 days after inoculation at the former temperature and in 12 days at the latter. At the two highest temperatures progressive wilting of the diseased plants was the most conspicuous symptom, while stem streaking and node browning were very slight or absent.

Host Range

Twenty-four species of the Leguminosae and six from other families were tested for susceptibility to the Wisconsin pea streak virus. Plants studied and results obtained are given in table 1. Recoverability of the virus from host plants was tested by inoculation back to healthy Perfected Wales peas. Although no symptoms were observed when the virus was

recovered, the degree of susceptibility was designated as "latent." If symptoms were absent or questionable and the virus was not recovered, the host was considered nonsusceptible. The virus was found to be limited in host range to the legumes, 17 species of which were infected. It was not infectious to cucumber (*Cucumis sativus* L.), jimsonweed (*Datura stramonium* L.), tomato (*Lycopersicon esculentum* Mill.), or any of the three species of *Nicotiana* tested.

TABLE 1.—*Host range and symptoms of the Wisconsin pea streak virus*

Host	Plants inoculated	Plants diseased	Outstanding symptom
	Number	*Number*	
Lathyrus hirsutus L.	18	Latent[a]	
L. odoratus L.	12	Latent	
Lupinus angustifolius L.	7	0[a]	
Medicago orbicularis All.	5	Latent	
M. sativa L.	17	0	
Melilotus alba Desr.	22	6	mottle
M. officinalis (L.) Lam.	22	9	mottle
Phaseolus vulgaris L.	32	0	
Pisum sativum L.	40	32	stem streak, plant wilt
P. sativum var. *arvense* Poir.	9	4	Do
Soja max (L.) Piper	31	12	very slight general chlorosis
Trifolium hybridum L.	24	13	faint mosaic
T. incarnatum L.	17	Latent	
T. pratense L.	40	32	mottle
T. repens L. (Ladino white clover)	22	2	very slight leaf wrinkle
T. repens L. (white clover)	18	0	
Trigonella foenum graecum L.	11	Latent	
Vicia atropurpurea Desf.	16	Latent	
V. faba L. var. *major*	9	0	
V. faba L. var. *minor*	14	0	
V. grandiflora Scop.	9	2	stem streak
V. monantha (L.) Desf.	10	6	Do
V. sativa L.	11	0	
V. villosa Roth.	9	Latent	
Vigna sinensis Endl.	18	0	
Cucumis sativus L.	37	0	
Datura stramonium L.	25	0	
Lycopersicon esculentum Mill.	7	0	
Nicotiana glutinosa L.	18	0	
N. rustica L.	4	0	
N. tabacum L.	14	0	

[a] Latent = no symptoms, but virus recovered; 0 = no symptoms and no recovery.

Reaction of Varieties of Pea and Bean

Eighteen pea varieties and twelve bean varieties were tested for their reaction to the Wisconsin pea streak virus. The varieties studied and the degree of their resistance or susceptibility are presented in table 2. In several tests with Wisconsin Perfection, it was found that no infection was obtained unless inoculation was made when the plants were very young—before the second set of leaves unfolded. All of the pea varieties tested were susceptible, and the virus was easily recovered from each of them. Diseased plants of Delwiche Commando, Horal, Loyalty, Merit, Pride,

Profusion, Wasatch, and Wisconsin Perfection showed more stem streaking and less early and permanent wilting than the other varieties tested. Symptoms were not induced on any of the bean varieties tested and in no case was the virus recovered.

Properties of the Virus

A series of experiments was conducted to determine the following physical properties of the virus: tolerance to dilution, resistance to aging *in vitro*, thermal inactivation, and longevity in frozen plant tissue. Table 3 shows the results obtained. This virus displayed a rather marked tolerance to dilution. In one trial it was found still infective after a dilution of 1:100,000; two subsequent trials showed that it could be diluted to

TABLE 2.—*Varietal reaction of pea and bean to the Wisconsin pea streak virus*

Pea variety	Plants inoculated	Plants diseased	Bean variety	Plants inoculated	Plants diseased
	Number	Number		Number	Number
Alaska	35	27	Bountiful	7	0
Alderman	28	7	Dwarf Horticultural	11	0
Bonneville	20	10	Great Northern		
Canner King	20	14	U. of I. No. 1	8	0
Delwiche Commando	16	7	Great Northern		
Glacier	23	13	U. of I. No. 59	5	0
Horal	35	15	Idaho Refugee	4	0
Improved Penin	13	6	Michelite	8	0
Loyalty	17	5	Pinto	11	0
Merit	19	17	Red Kidney	32	0
Perfected Wales	39	28	Sensation Refugee 1066	9	0
Pride	16	5	Stringless Blue Lake		
Profusion	22	10	(Black)	4	0
Resistant Famous	31	22	Stringless Blue Lake		
Thomas Laxton	28	12	(White)	4	0
Wasatch	15	3	Stringless Green		
Wisconsin Early Sweet	28	21	Refugee	7	0
Wisconsin Perfection	19	2			

1:1,000,000 and still cause infection. One experiment indicated that the virus was still infectious after aging for eight days. In the other trials, when aging studies were lengthened to include 64 days, it was found that this virus withstood aging between 16 and 32 days. All three thermal inactivation trials showed that the virus was inactivated after a 10-min. exposure at 60° C. The thermal inactivation point was somewhere between 58° C. and 60° C. When diseased plants were frozen for periods of up to 160 days, one trial showed that this virus could withstand 40 days of freezing, while the two other trials indicated survival up to only 10 and 20 days, respectively.

Insect Transmission

The possibility of the ability of the pea aphid (*Illinoia pisi* (Kalt.)) to transmit the virus was studied in the greenhouse. In all these studies the

aphids, after feeding on diseased plants, were allowed a 2-day feeding period on healthy plants. No infection was obtained with the pea streak virus in any of 5 trials, but tests made simultaneously with pea mosaic and pea stunt viruses yielded successful transmission. No transmission was secured in further tests in which aphids were allowed to feed on diseased plants for periods ranging from 15 min. to 21 days. Starving aphids

TABLE 3.—*Physical properties of the Wisconsin pea streak virus*

Property test	Trial 1		Trial 2		Trial 3	
	Plants inoculated	Plants diseased	Plants inoculated	Plants diseased	Plants inoculated	Plants diseased
Dilution:						
0	12	5	22	21	10	8
1 : 10	22	15	22	21	26	22
1 : 100	26	17	20	17	25	4
1 : 1,000	20	6	23	15	19	10
1 : 10,000	23	0	24	3	28	2
1 : 100,000	25	1	18	0	25	1
1 : 1,000,000			22	2	20	5
Aging *in vitro:* (days)						
0	10	2	11	5	11	8
1	11	8				
2	12	5	9	4	10	4
4	11	4	10	5	9	4
8	12	1	9	3	11	9
16			10	2	9	7
32					10	0
64					11	0
Heat: (° C.) (10 min.)						
0	24	17	9	6	9	4
54					10	4
56	23	5	11	1	9	3
58	24	1	11	2	9	3
60	26	0	11	0	10	0
62	22	0	12	0	9	0
64	25	0	10	0	11	0
Freezing: (days)						
0	10	10	10	10	10	7
5	7	6	10	3	10	7
10	10	0	10	3	9	5
20	10	4			6	2
40	10	1			11	0
80	13	0			10	0
160	11	0	9	0		

for 4 and 8 hr. before feeding on diseased plants likewise had no effect. In one such test the insects were starved for 4 hr. and allowed a 2-day feeding period before transfer to healthy plants. After the 8-hr. starving period, aphids were allowed to feed for 15 min., 45 min., 1½ hr., and 12 hr. before removal to healthy plants. These manipulations also yielded negative results, as did transmission tests when winged aphids were used. Limited tests with the peach aphid (*Myzus persicae* (Sulzer)) also gave negative results.

When field transmission of the virus was to be studied, Perfected Wales peas were grown in 6-in. rows in a 12 × 24-ft. plot, and mechanical inoculation of one plant near the center of plot was made. Fourteen pea aphids were placed on this plant when streak symptoms first became apparent. At this time two naturally occurring pea streak infection centers were noted toward the eastern end of the plot. Each consisted of five or six diseased plants. No other virus disease symptoms could be seen, even on the plants adjacent to the one mechanically inoculated. Twenty-one days after inoculation the mechanically inoculated plant was dead. Plants within a radius of 6 ft. of this inoculum plant were closely examined, but did not show a higher percentage of infection than those in the outlying areas of the plot where random counts revealed from 8 to 13 diseased plants per 100 examined. Two exceptions were noted, viz., the natural infection centers mentioned above, and an area about 4 × 16 ft. along the southern edge of the plot where only one out of 100 plants was diseased. Twelve days later random counts were made at each end of the plot. At this time 21 plants per 100 were diseased at the western end, and 29 plants per 100 were infected at the opposite end. Pea aphids were abundant throughout the plot at all times. The distribution of the diseased plants in the plot did not indicate that the virus was spread by the pea aphid, but it did indicate clearly that probably some insect was responsible for its distribution.

Seed Transmission

Only limited seed transmission trials were possible because most pea plants infected with the virus developed only distorted pods containing very few viable seeds or were killed before pods matured. Plants were grown from 211 seeds collected from diseased plants. Under optimum greenhouse conditions for disease development, no symptoms developed on any of these plants.

DISCUSSION

To support the conclusion that the Wisconsin pea streak virus is different from previously reported pea streak viruses, a comparison will be made of the experimental evidence just presented with former descriptions of other viruses inciting pea streak.

The American pea streak virus reported by Zaumeyer (13) in 1938 differs considerably from the virus just described. Zaumeyer's virus, pea streak virus 1, showed less resistance to aging in vitro (2 days), a higher thermal inactivation point (65° C.), and lower tolerance to dilution (1:5,000); and it was infectious to white clover, alfalfa, and broad bean but not to sweet pea. The foliage distortion, apical rosette, and vein prominence described as symptoms were not observed on plants infected with the Wisconsin streak virus. Pea streak virus 1 was transmitted by the pea aphid, but no success was achieved in attempts to transmit the Wisconsin virus by that means.

Chamberlain (1) described the New Zealand pea streak virus in 1939 and called it *Pisum* virus 3. It differs from the Wisconsin pea streak virus in symptom expression (local lesions on inoculated pea leaves, apical plant wilt); in resistance to aging *in vitro* (41 days); in thermal inactivation point (80° C.); and in host range, since it was infectious to blue lupine, kidney bean, white clover, and cucumber.

The pea wilt virus described by Johnson (3) is similar to the Wisconsin pea streak virus in physical properties, but differs in symptoms on peas, in being infectious to kidney bean, white clover, cowpea, and broad bean, and in being noninfectious to soybean.

Other viruses have been found to produce necrosis and streaking of peas, but they differ from the Wisconsin pea streak virus in symptom expression and in host range. In this category the following may be noted: cucumber virus strains 14 and 17 of Whipple and Walker (12); tobacco ringspot virus (9); tomato spotted wilt virus (7, 8, 11).

One of the striking aspects of the Wisconsin pea streak virus is its wide annual prevalence in Wisconsin pea fields in spite of the fact that the pea aphid is not a vector. Since red clover, Ladino white clover, white sweet clover, and yellow sweet clover are susceptible, there is no lack of possible overwintering reservoirs. Further study is needed to determine the means of its current-season dissemination.

SUMMARY

Wisconsin pea streak and the causal virus are described. The most obvious symptoms on pea in the field are a necrotic streaking of the stems and petioles and node browning. The characteristic symptoms in the greenhouse were browning at the node and wilting of the plant. Low temperature (16° C.) delayed symptom expression, and necrotic stem streaking and node browning were more typical at that temperature, while wilting was the most striking symptom at 24° and 28°. The virus was limited to the Leguminosae. All 18 pea varieties tested were susceptible but none of the 11 bean varieties inoculated was infected. The virus was still infective at a dilution of 1:1,000,000 and resisted aging *in vitro* between 16 and 32 days. Its thermal inactivation point was between 58° and 60° C. and it could withstand freezing *in vivo* for 40 days. The pea aphid was not found to be capable of transmitting the virus, and seed transmission was not observed.

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STIMULATED PYCNIDIUM PRODUCTION AND SYMPHOGENOUS PYCNIDIA IN PHOMA LINGAM[1]

O. H. CALVERT AND GLENN S. POUND[2]

During a study of induced variability in the Puget Sound strain of *Phoma lingam* (Fr.) Desm. (2), an unusual phenomenon occurred in a week-old Petri plate containing several single-spore colonies derived from non-treated spores of the original isolate described by Pound (7). Heavy lines of pycnidia developed between neighboring colonies of rather strikingly different types. One of these types, herein referred to as type A, was characterized by a comparatively slow growth rate, moderate to scant aerial mycelium, and few or no pycnidia. The other type, herein referred to as type B, was characterized by a fast growth rate, abundant aerial mycelium, and heavy production of pycnidia, usually in clumps. Later, many induced mutants of the Puget Sound strain were found to agree with type A in their reaction when paired with the original A and B isolates. The type A cultures differed considerably among themselves, whereas the type B cultures were all very similar and essentially unchanged from the original isolate of the strain. Since all B cultures were identical with the original isolate and gave no reaction when paired with it, the A cultures are all considered as mutants. No natural variability of the Puget Sound strain of *P. lingam* was observed by Pound (7), but the occurrence of the A type in a transfer of the original culture (which was derived from a single uninucleate spore) can only be explained as due to mutation. Several isolates of each type were studied in an effort to determine the cause of the heavy pycnidium production in the merger zone between colonies of type A and type B. From the data presented herein, it is thought that a symphogenous production of pycnidia (pycnidia produced from hyphal branches of several mycelial threads) occurs when A and B mycelia merge, but that meristogenous production of pycnidia (pycnidia produced from a single cell or adjacent cells of a single hypha) occurs within each mycelium.

MATERIALS AND METHODS

Of the isolates studied, eight mutant isolates (A2, A5, A6, A7, A14, A15, A18, A19) and four normal isolates (B2, B5, B14, B21) originated from single conidia taken from the untreated spore suspension of the original

strain. Six other mutant isolates (A3, A4, A9, A10, A12, A22) and two normal isolates (B12, B22) originated from single conidia taken from a spore suspension treated for 2 min. with a 0.02 mM per ml. concentration of nitrogen mustard, methyl-bis (beta-chloroethyl) amine.

Isolates were grown on potato-dextrose agar, a single lot of agar being made for each experiment. Germinating single spores were picked with a needle from water-agar plates under a stereoscopic microscope. In the pairing experiments, single spores of monoconidial lines were used. In assaying the population of single pycnidia, each pycnidium was crushed in a drop of sterile water, and a loop of the suspension was transferred to 40 ml. of water which was then poured over the surface of agar plates. When the spores were germinating they were lifted, individually, and transferred to test-tube slants. After a few days they were separated into A and B types, according to their cultural type. As a confirmation of the type diagnosis, representatives were plated out in pairs to observe the reaction between them. In addition, some plates seeded with the spore suspension from single pycnidia were allowed to incubate so that the reaction between neighboring colonies could be observed. Confirmation tests showed that, in all cases, the separation of the spores into the two groups was accurate.

RESULTS OF PAIRING A- AND B-TYPE ISOLATES

The 20 cultures of the A and B types were paired in all possible combinations. Eight transfers, in regular patterns, were made to Petri dishes so that each isolate was grown beside each of the other isolates. These data show that A-type isolates, although they varied considerably among themselves (Fig. 1) in regard to growth rates, sporulation, pigmentation, etc., were constant in their reaction to the B-type isolates, which were all very similar. In each case a heavy line of pycnidia was produced in the zone of merging hyphae between an A and B isolate (Fig. 2). This was never true when two A isolates were paired, or when two B isolates were paired (Fig. 2).

Several of the A and B isolates were inoculated to cabbage, where they produced symptoms identical with those produced by the original strain. Reisolations from cabbage yielded cultures which gave identical reactions to the respective original A and B isolates. Thus, in passage through host tissue, the isolates were not changed in their reaction to each other.

RESULTS OF SAMPLING SPORES OF SINGLE PYCNIDIA

To determine what type spores the individual pycnidia from an A-B merger zone contained, several single pycnidia of an A9-B22 pairing were crushed, diluted, and plated out. Usually, the diluted suspension was kept in a refrigerator for 24 hr. while a sample plate seeded with it was incubated in order to determine the extent of dilution required. By this method the desirable population of spores per plate was easily obtained. The re-

sults are summarized in table 1.   In practically every case, pycnidia from
the merger zone of two different colonies yielded both A and B types (Fig.
2, E, F).   Care was always taken to select pycnidia before spores were dis-
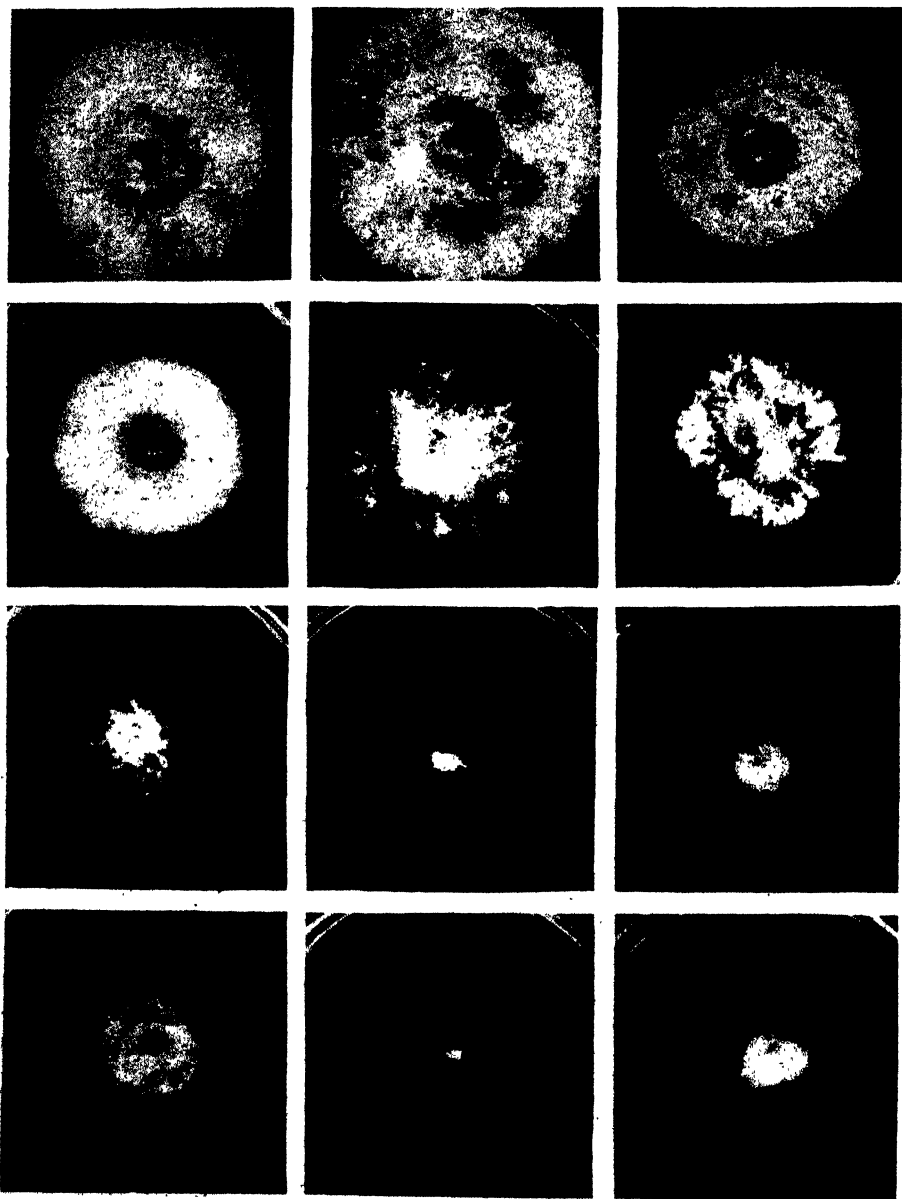
FIG. 1. Several A isolates of the Puget Sound strain of *Phoma lingam* showing differences in cultural characters.   All gave identical reactions to B isolates.

charged and to choose those that were isolated so that the possibility of con-
taminating spores from other pycnidia was eliminated.   Occasionally only
one type was recovered (*e.g.*, pycnidia 3 and 6).   This, however, is to be
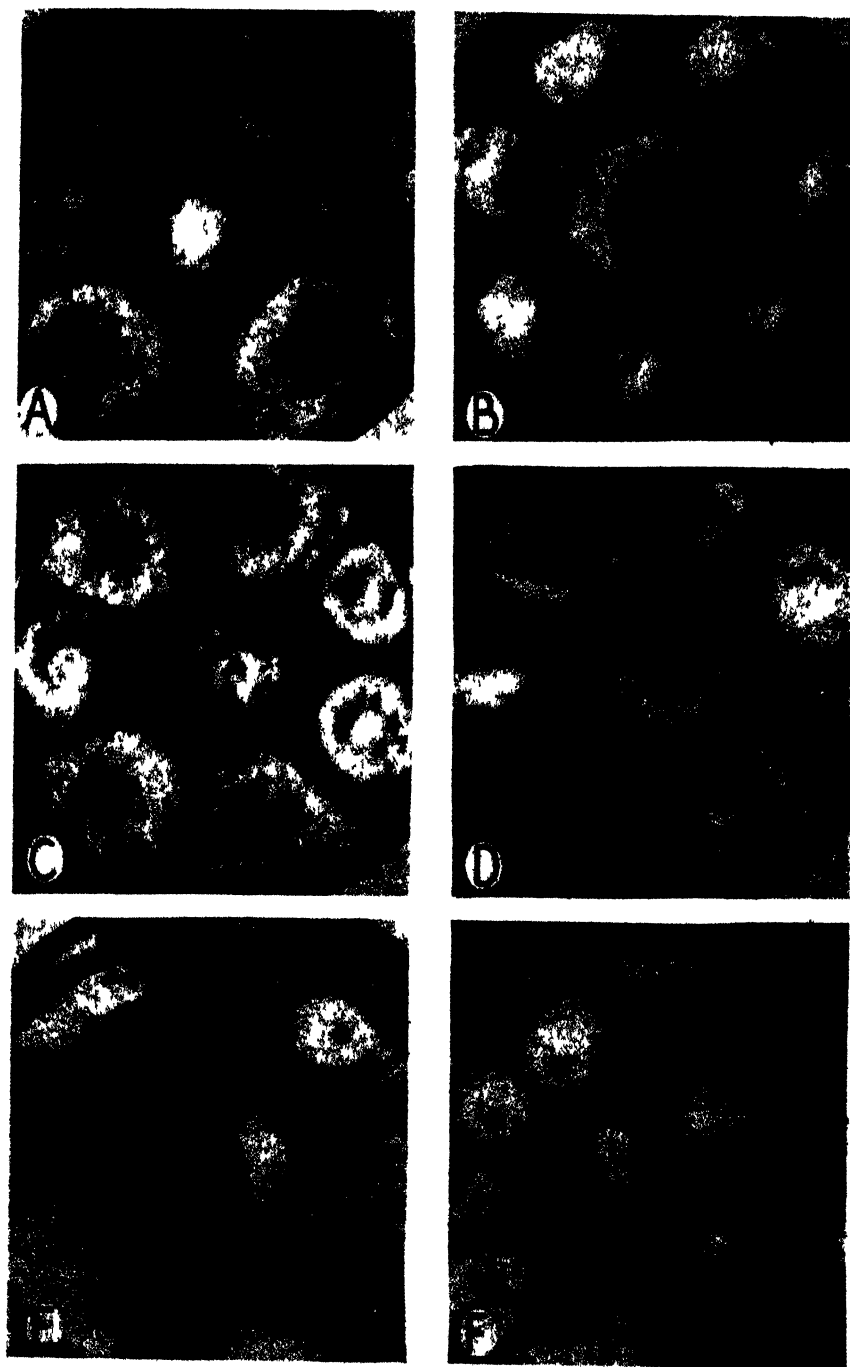
FIG. 2. Methods and results of pairing A and B isolates. A. Peripheral colonies are B22; central colony, A9. B. Peripheral colonies are A4; central colony, B5. C, D. Four largest colonies are B22; smallest, A9 and A4. E, F. Plates seeded with spores from a single pycnidium of an A-B pairing. Note heavy pycnidial lines between colonies of opposite types.

expected since both isolates sporulated to some extent and there was always the possibility of isolating a pycnidium produced by either isolate rather than by both together. Control pycnidia, however, which were in each case obtained from interior portions of the colony, invariably yielded only spores identical with the respective parent isolate. In many cases the two types were recovered in almost equal proportions, while in others one type predominated.

Since pycnidia containing both A- and B-type spores were produced by pairing uninucleate conidia of the two types, it seemed probable that one

TABLE 1.—*Frequency of spore types obtained from single pycnidia produced by pairing A and B-type isolates of the Puget Sound strain of Phoma lingam*

Strain combination and pycnidia	Total spores isolated[c]	Number of spores of type A	Number of spores of type B	Reaction when re paired[d]
A9-B22[a]				
1	164	86	78	HPL
2	238	186	52	HPL
3	92	92	0	0
4	68	32	36	HPL
A9 control	100	100	0	0
B22 control	94	0	94	0
5[b]	1630	860	770	HPL
6	113	113	0	0
7	140	120	20	HPL
8	960	260	700	HPL
9	1060	380	680	HPL
10	219	146	73	HPL
11	236	180	56	HPL
12	3620	1820	1800	HPL
13	200	146	54	HPL
A control	100	100	0	0
B control	100	0	100	0
14	310	170	140	HPL
15	205	62	143	HPL
16	240	105	135	HPL
17	187	57	130	HPL
A control	200	200	0	0
B control	200	0	200	0

[a] This original pairing was of 2 single conidia.

[b] Pycnidia 5 through 13 were taken from the pycnidial line between pairings of single spores from pycnidium 1. Pycnidia 14 through 17 were taken from the pycnidial line between pairings of single spores from pycnidium 5.

[c] In most cases single spores were transferred to test-tube slants and diagnosis made on cultural type but confirmed by re-pairing representative isolates. In some cases spores were counted on Petri plates and diagnosis made according to cultural type and reaction.

[d] HPL = heavy pycnidial line; 0 = no reaction.

of two things was happening. First, since hyphal cells are multinucleate, nuclei of the two types, within the same cell as a result of anastomosis, might participate in the formation of a pycnidium. Since the spores are uninucleate, segregation would occur and both types would be reproduced in the formation of pycnospores. The heavy sporulation then could be due to heterocaryotic vigor (1, 3). Secondly, the two mycelia might participate

in a physical way in the formation of a single pycnidium and each would abstrict its own conidia within the pycnidium. To test which of these possibilities was correct, experiments detailed in the following section were performed.

EFFORTS TO ISOLATE HETEROCARYOTIC MYCELIUM

Thirty-six pycnidia from the merger zone of an A-B pairing were isolated individually and planted in Petri plates so that mycelium growing from one could react with that of other pycnidia and with A9 and B22. If the pycnidia contained both types of spores, as has been shown, the mycelial growth from the pycnidial wall would also contain either (a) heterocaryotic cells from which both types could be recovered by hyphal tip analysis, or (b) hyphal strands of A and B mycelia. In practically every case, the mycelia from the single pycnidia reacted with mycelium of A9 as if they were B cultures. Only occasionally did they react with B or with each other. In this case there was obvious separation of the colonies into areas in which the A mycelium predominated and those in which the B mycelium predominated. It was only the segments of the A mycelium that reacted with B or with mycelium of other pycnidia. However, in each colony there was obvious interaction between A and B mycelia. In the central portion of the colonies, pycnidia were laid down in heavy lines, in an irregular, dendritic pattern not typical of either A or B or of a random pattern. Samplings from these individual pycnidia always yielded both A and B spores. Furthermore, the portion of the colony bearing the pycnidia agreed very closely with the diameter of colonies of A9 alone. The fact that each of the pycnidial colonies reacted with A9 but not with B or with each other and that individual pycnidia yielded both types of spores is interpreted as indicating that the peripheral mycelium was that of B22 alone, since it is much faster-growing than that of A9. Twenty-five hyphal tips were isolated from the periphery of some of the pycnidial colonies. Spores sampled from pycnidia produced by the hyphal tip cultures in each case were of B type only.

In a similar experiment, spores of A9 and B22 were mixed in equal amounts in a heavy suspension and a loop of the mixture placed in the center of a Petri plate. It was thought that the germ tubes of germinating spores might anastomose and a heterocaryotic mycelium would grow out from the seeding. The same situation occurred as described above in the pycnidial colonies. The region of heavy sporulation agreed very closely with the diameter of growth from a loop of A9 spores alone and the pattern of pycnidial formation indicated reacting mycelial strands. Samplings of these individual pycnidia in every instance yielded both types of spores. Hyphal tips were taken from the mixed culture as soon as possible, and out of 33 there were 7 of the A type and 26 of the B type. In no case were both types recovered in the progeny of a single hyphal tip. Peripheral growths of colonies from pycnidia taken from the merger zone of an A-B pairing

and from reacting mycelial strands of an A-B spore mixture were macerated in a Waring Blendor and then poured over agar plates. Of 120 hyphal fragment cultures thus established, all were of the B type. The progenies of these cultures were identical with the B parent and in no case were both types found in the progeny of a single hyphal fragment. Samplings of individual pycnidia from the same source as those from which the peripheral growth was taken always yielded both types of spores.

The progeny of 145 single spores picked at random from a single pycnidium from the merger zone of an A-B pairing were tested to determine if, perchance, any spores could be isolated which contained both nuclear types. This would be expected if the mycelium was heterocaryotic and if the spores ever had more than one nucleus. In each case, spores of type A yielded only type A colonies and those of type B yielded only type B colonies. No third type was found. Although this did not indicate whether or not heterocaryosis occurred, it would confirm the uninucleate condition of the spores as stated by Calvert *et al.* (2) if heterocaryosis did occur.

The repeated recovery of both A and B spores from a single pycnidium and the failure to demonstrate heterocaryosis by hyphal tip analysis indicate that the A and B mycelia when they are in contact participate mutually in the production of pycnidia. These "conjuncture" pycnidia do not differ in appearance from those produced by the B mycelium except that they are laid down in heavy lines rather than in clumps as for B isolates.

MORPHOLOGY OF PYCNIDIUM PRODUCTION

Colonies developing from single spores of A9 and B22 were observed carefully under a microscope to determine the type of pycnidium development. With each strain, development was meristogenous. A single cell or a few adjacent cells enlarged and branched profusely. These fingerlike branches intertwined and apparently fused to ultimately form a pycnidium. In no instance was symphogenous development found. The type of development for the pycnidia in the reaction line could not be ascertained because of difficulty in observing them. Strands of A mycelium met the hyphae of the B mycelium and penetrated the B growth for 3 or 4 millimeters before any reaction occurred. The pycnidial development was shielded by B mycelium and observation was thus made difficult. If the two mycelia participated mutually in forming individual pycnidia, the development would be symphogenous and different from that common in each isolate. Kempton (6) also found only meristogenous development in the *Phoma* species which he tested. He also demonstrated, however, that some species of fungi may produce pycnidia by either or both methods.

RESULTS OF PAIRING A AND B ISOLATES WITH OTHER STRAINS OF PHOMA LINGAM AND OTHER SPECIES OF PHOMA

Isolates A9, B22, and the S1, S39, W11, and Iowa 11 strains of *Phoma lingam* described by Pound (7), and *Phoma betae* (Oud.) Fr. and *Pyreno-*

chaeta terrestris (Hansen) Gorenz *et al.* (*Phoma terrestris* Hansen) were paired in all possible combinations in Petri plates. A9 gave no reaction with any isolate other than B22 except for an occasional dark aversion line with S1 and S39. All three of these isolates are slow-growing and normally produce staling products. Isolates S1 and S39 gave no reaction with any isolates except B22 with which they produced definite dark aversion lines. Their mycelial growth was stopped and darkened upon contacting that of B22. Iowa 11 and *Pyrenochaeta terrestris* gave no reaction with any isolates. W11 caused a slight but definite production of aerial pycnidia in the mycelium of B22 on the side of their contact. Isolations from these individual pycnidia always yielded B spores alone. A similar and more marked reaction was produced on B22 by *Phoma betae*. Upon contact of the two mycelia, a heavy line of pycnidia was laid down in the medium by B22, and a profuse production of aerial pycnidia occurred in the B mycelium
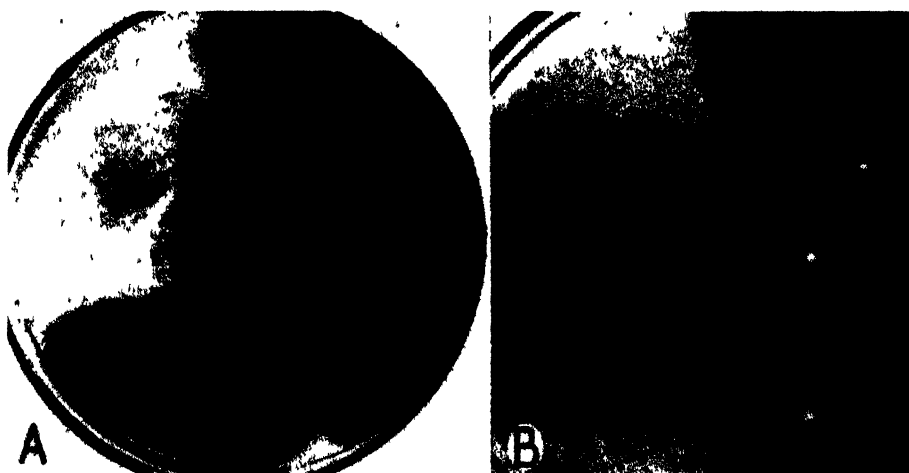
FIG. 3. Inducement of sporulation in the B22 isolate of *Phoma lingam* by *Phoma betae.* A. Profuse development of aerial pycnidia in B22 (left) when grown beside *P. betae* (right). Note that *Pyrenochaeta terrestris* (bottom) had no effect on B22. B. Heavy line of pycnidia laid down in the medium by B22 (left) when grown beside *Phoma betae.*

on the side of contact with *Phoma betae* (Fig. 3). Isolations from both types of individual pycnidia always yielded only B spores. This indicates that *Phoma betae* produced a substance stimulating pycnidium production in B22 rather than a symphogenous reaction like that of A9, although the two reactions appeared very similar in the Petri plates.

In a single experiment, cultures of A9 and B22 and B22 and *Phoma betae* were set up in U-tubes of 6-in. length such that the paired isolates were separated by agar of sufficient depth to prevent the mycelium of one contacting that of the other. Control tubes contained only isolate A or B in only one arm of the tube. After the yellow pigmentation in the control

tubes had diffused throughout the medium, notes were taken on the paired cultures. In no case was there evidence of increased sporulation in either A or B when paired with each other. This would indicate that the increased sporulation in the zone where the two mycelia meet is probably not due to a chemical substance which is diffusible through the medium. In the U-tubes containing B22 and *Phoma betae,* there was apparent increase in sporulation in the B22 colonies. This would indicate that *Phoma betae* does produce a diffusible substance which stimulates pycnidium production in B22.

DISCUSSION

It is believed that sufficient evidence is presented to indicate that the ridge of pycnidia produced between an A and a B isolate of *Phoma lingam* and described herein is not due to heterocaryotic vigor nor just to stimulation of sporulation in one strain by the other. The failure to isolate heterocaryotic mycelium by hyphal tip analysis and the absence of an increase in growth rate over that of B cultures alone indicate that heterocaryosis is not responsible for the increased sporulation. Also, the repeated recovery of both A and B types from single pycnidia indicates that it is not just a case of one strain stimulating pycnidium production in the other. It is believed that the line of pycnidia produced beween an A and a B isolate results from a mutual physical interaction of the two mycelia without anastomosis followed by nuclear migration. Thus, each type of mycelium abstricts it own uninucleate spores within a symphogenous pycnidium.

A reaction whereby two distinct strains of a fungus participate mutually to form a single pycnidium without heterocaryosis has been described for only a very few fungi. Goossens (5) described a condition in *Phoma apiicola* Klebahn which was apparently an identical counterpart to that described in this paper. He found that if he paired the microform and the macroform spores of *P. apiicola* in Petri plates, a heavy line of "conjuncture" pycnidia was produced in the zone of merging hyphae. Samplings of individual conjuncture pycnidia yielded spores of both types. Goossens' interpretation of the phenomenon was that both mycelia participated in the formation of the pycnidia, each abstricting its pycnospores within. Das Gupta (4) described the production of pycnidia in *Cytosporina ludibunda* Sacc. by the intermingling of two nonsporulating strains. He gave no explanation of the reaction but was able to recover both strains from single pycnidia.[3]

Although this reaction is not a common one, it has some bearing on the question of variability. It is not an example of variability but rather a result of variability. However, isolating two distinct strain types from the spore progeny of a single pycnidium would certainly lead one to suspect either a heterocaryotic condition of the mycelium, or mutation. In this instance, neither is the case.

[3] Unpublished communication.

SUMMARY

A reaction between two distinct types (A and B) of the Puget Sound strain of *Phoma lingam* in which a heavy line of pycnidia is produced in the zone where the two mycelial types merge is described. Samplings of spores of individual pycnidia taken from this merger zone almost always yielded both cultural types. Repeated efforts to prove a heterocaryotic condition by hyphal tip analysis failed. It is thought that the two mycelia participate mutually in the formation of pycnidia, each abstricting its pycnospores within. Passage of the two types through host tissue did not alter their reaction toward each other. Isolates of type A vary considerably in cultural characters but are identical in their reaction with type B isolates. Type B isolates were all identical and unchanged from the original Puget Sound isolate.

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REPORT AND ABSTRACTS OF THE THIRTY-FIRST ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The 31st annual meeting of the Pacific Division of The American Phytopathological Society was held at the University of British Columbia, Vancouver, British Columbia, June 16–18, in conjunction with the meeting of the Pacific Division of the A.A.A.S. Approximately 80 persons attended the meeting, with Alberta, British Columbia, Manitoba, Ontario, Quebec, England, California, Hawaii, Michigan, Minnesota, Oregon, Washington, and Wisconsin represented. A number of members of the Canadian Phytopathological Society were in attendance. Included on the program were 30 papers, four discussion sessions, a banquet, and a field trip. Topics for the discussion sessions were "Virus Strains, Mutation, and Acquired Immunity," "Soil Factors in Relation to Root Diseases," "Hybridization and Mutation in Fungi," and "Developments in the Field of Fungicides." Dr. E. C. Stakman addressed the banquet on "Evolution of Plant Pathology." A field trip to the Fraser River Valley concluded the meeting on June 18.

The following are officers for the calendar year 1950:

President: William C. Snyder
Vice-President: Earle C. Blodgett
Secretary-Treasurer: George A. Zentmyer ·
Councilor: L. C. Cochran

ABSTRACTS OF PAPERS PRESENTED AT THE MEETING

Control of walnut blight by sprays in 1947 and 1948. ARK, PETER A., and ROBERT S. DICKEY. Pollen dissemination of walnut blight, coupled with heavy inoculum from oversummering blighted leaf buds, presents a difficult problem in blight control. Bordeaux mixture, although possessing good germicidal and weathering properties, has been found hazardous under certain conditions in California. In 1947 and 1948 the following materials were tested to control walnut blight: ammoniacal copper, yellow cuprocide, red copper oxide, basic copper sulfate, copper hydroxide, copper carbonate, Dithane D-14, Isothan Q15, Fermate, Puratized, Phygon, copper A, copper oxychloride sulfate (COCS), Helione (dihydrochloride salt of aminoazo-benzene), and streptomycin. In all trials one pre-bloom spray and one post-bloom spray were applied. It appears that ammoniacal copper (containing from 0.3 to 0.6 per cent of metallic copper in a final spray), copper A (1.35 per cent metallic Cu in a final spray), and yellow cuprocide (1.66 per cent metallic Cu in a final spray) give good practical control of the disease with good increase in yield, in comparison with unsprayed trees, and do not induce visible injury.

Hot-water treatment of orange trees for eradication of the citrus-root nematode and Phytophthora citrophthora. BAINES, R. C., L. J. KLOTZ, O. F. CLARKE, and T. A. DeWOLFE. The citrus-root nematode, *Tylenchulus semipenetrans* Cobb, was eradicated from the roots of young, bare-root, sour orange trees by submerging the roots in water at 113°, 116°, or 119° F. for 25, 10, and 5 min., respectively. The nematode also was eradicated from sour orange trees in pots by heating the soil and roots at 115° F. for 20 min. in a water bath. Heating the soil and roots of balled orange trees at 102°–105° F. for 20 hr. in moist air eradicated the citrus-root nematode and *Phytophthora citrophthora* (Sm. and Sm.) Leon. infections. The temperature of the soil of the potted and balled trees lagged and required 2.3 to 3 hr. to reach the desired temperatures. Injury of the roots occurred from heating bare-root trees at 116° F. for 20 min., 119° F. for 10 min., and 121° F. and above for 5 min.

Lemon tree collapse. CALAVAN, E. C. This disease of lemon trees, which outwardly resembles quick decline of oranges, has been studied for three years in southern California. Collapse follows a prolonged period of starvation of the rootstock and generally occurs between December 15 and June 1. External symptoms are a twisting leafroll followed by slight to extreme wilt, excessive fruiting, small and prematurely colored fruit, lack of growth, sloughing of bark from rootlets, and, frequently, yellowing of the foliage and partial defoliation. Lemon collapse is most common on, but not confined to, grapefruit and sweet orange rootstocks. Shell bark may or may not be present. Trees may die soon after wilting or may establish an equilibrium and partially recover. Severe pruning often stimulates new growth in collapsing trees but this improvement is usually tempo-

rary. Bacteria and fungi, including *Pythium ultimum* and *Fusarium solani*, associated with the roots of diseased trees are common also on the roots of normal trees. There is no evidence of natural spread in the field. Transmission tests by budding have produced no positive reaction in 13 months. Differences in strain susceptibility have been observed. The Eureka variety appears most susceptible.

Preliminary studies on Agrobacterium (Phytomonas) rubi Hildebrand. COLE-MAN, L. C. *Phytomonas rubi* erected by Hildebrand in 1940 and characterized as exclusively attacking canes of *Rubus* species is a common parasite on Himalaya blackberry on Vancouver Island. In an attempt to find suitable material for a cytological study of plant tumors, this organism was isolated and inoculated successfully into stems of *Vicia faba.* Attempts to infect this host with *Phytomonas tumefaciens* had failed. While *P. rubi* is nonvirulent on most of the hosts of *P. tumefaciens* recently used for experimental purposes, sunflower is an exception. On this host the reaction to the two species is very similar, *P. tumefaciens* usually inducing a larger growth. A striking difference is the epinastic reaction of leaves with *P. tumefaciens,* which is entirely lacking with *P. rubi.* On sunflower, incipient galls of *P. tumefaciens* do not prevent a bending reaction with indoleacetic acid, while those of *P. rubi* do prevent bending. Similarly, incipient galls on *V. faba* prevent the typical bending reaction with indoleacetic. These phenomena are probably associated with a differential production of auxin inactivating enzymes. Galls produced by *P. rubi* on blackberry are not confined to the canes. It has been so far impossible to infect either Himalaya blackberry or red raspberry with *P. tumefaciens.*

Etiology and control of Sclerotina sclerotiorum on Blue Lake beans. DANA, B. F., and EDWARD K. VAUGHAN. Localized minor losses from *S. sclerotiorum* in miscellaneous crops become locally of major importance under intensive irrigated culture of Blue Lake beans for processing. Direct infection of leaves by ascospores from apothecia was obtained in a closed chamber. Apothecia were found to mature the year around with favorable moisture and temperatures above freezing. In field plots randomized and replicated four times both dust and spray applications of fungicides were made to Blue Lake beans in trellis culture. Copper-sulfur and bentonite sulfur as dusts and Zerlate spray reduced infection centers from ⅓ to ½ the number in untreated plots. Bismuth subsalicylate spray cut infection centers to $\frac{1}{10}$ the number in untreated plots. Similar reduction in dead plants was obtained with these field treatments. Bioquin reduced infection centers but caused some plant injury and skin irritation to workers. Infection centers in plots treated with Parzate, Phygon, and silver nitrate were much more numerous than those in untreated plots. These data are from plots in two locations and on crops grown in 1948.

Injury caused by treating tomato seed with mercurials. DICKEY, ROBERT S., and PETER A. ARK. In greenhouse tests $HgCl_2$ and New Improved Ceresan caused a considerable depression of germination in tomato seeds (var. Pearson) when the ratio of the seed to the treating solutions is 1 to 300 and the duration of the treatment from 10 to 15 min. and readings on germination taken for 32 days. Treatment for 10 min. with $HgCl_2$ 1:1000, resulted in 25 per cent germination when the treated seed was not washed and 70 per cent germination when the seed was washed for 15 min. With $HgCl_2$ 1:2000, unwashed seed yielded 68 per cent germination, washed seed 84 per cent. With $HgCl_2$ 1:3000, unwashed seed germinated 89 per cent, washed seed 93 per cent. Untreated seed germinated 90 to 98 per cent. Treatment with New Improved Ceresan resulted in the following percentages for the treated (10 min.), treated (10 min.) and washed (15 min.), and untreated lots: 1:1250—50, 83, 95; 1:2500—82, 93, 95; 1:3750—93, 95, 98. Seed treated with higher concentrations of either $HgCl_2$ or New Improved Ceresan should be washed in water for at least 15 min.

Effect of streptomycin on plant cells. DUFRENOY, JEAN, ROBERTSON PRATT, and VIRGINIA L. PICKERING. Cells of epidermal strips of various plants remain alive and turgid and seem normal for 2 to 3 days in solutions containing streptomycin and sugar, whereas they deteriorate rapidly in sugar solution alone. The results cannot be interpreted as due merely to protection from bacterial contamination. Mitochondria tend to elongate in cells exposed to streptomycin. Evidence is presented which suggests that (1) streptomycin does not adversely affect desoxyribonucleic acid derivatives involved in the nuclear structure of plant cells, and that (2) it may adversely affect chlorophyll-bearing cells by linking with ribonucleic acid. Streptomycin appears

to be of potential value in cytochemical or tissue culture investigations, since it permits prolonged survival of tissues in cultures, simultaneously protecting them against a number of bacterial contaminants.

Studies of some curly-top virus strains. GIDDINGS, N. J.

Studies on control of snow mold of winter wheat in Washington. HOLTON, C. S., and RODERICK SPRAGUE. Snow mold caused by *Typhula idahoensis* Rensb., *T. itoana* Imai, and *Fusarium nivale* (Fr.) Ces. has caused yearly losses in Douglas and Lincoln Counties, Wash., ranging from $25,000 to $300,000. The following practices have been found by us to reduce losses: August seeding instead of September, while otherwise undesirable, is necessary to permit formation of large roots and crowns. Such plants, even though all the leaves are killed, will usually recover enough so that reseeding in the spring is not necessary. Mold-injured stands must be sprayed with herbicides to supress weeds. Fertilizers, including pelleted ammonium phosphate and ammonium nitrate, applied in the fall, have no appreciable effect on the causal organisms, but small quantities, up to 60 lb. per acre, tend to aid recovery from mold after the snows leave. Black superphosphate or lamp black at 500 lb. per acre applied on snow in March 1948 and in 1949 aided in melting the snow and checked activity of the molds. Spergon, Phygon, and Puraturf applied at 80 lb. per acre in mid-October resulted in 95 to 100 per cent control in 1948 and 1949.

Blackberry rust in the Pacific Northwest. JOHNSON, FOLKE, and GEORGE W. FISCHER.

Limitations of the hot-water treatment for the control of brown rot of lemons. KLOTZ, L. J., and T. A. DE WOLFE. For adequate control of brown rot of lemons in the packing house an immersion of 4 or more minutes in water at 118°–120° F. is neces sary. This treatment will arrest decay in lemons that had been infected as long as 60 hr. previously if the temperature of the fruit had not gone above 54° F. before treat ment. To avoid rind oil injury, cold, turgid lemons should be wilted slightly before immersion in hot solution. The nearer the orchard temperature is to the optimum growth temperature of the fungus (78°–80° F.) during and just following the win ter and spring rains, the greater the number of infected lemons and destructiveness of brown rot. The rate of penetration and decay of lemons by the fungus is more rapid in mature than in immature fruit. Cold solutions of fungicides, such as a 1:1000 solution of copper sulfate or hypochlorite solutions, were ineffective in stopping decay.

Pathological effects of curly-top virus on dodder. LACKEY, C. F., and C. W. BEN NETT. When *Cuscuta subinclusa* or *C. californica* parasitizes plants affected by curly top they acquire virus apparently in concentrations about equal to those of their host plants. However, when branches of dodder are removed from the diseased plants and established on plants from which no additional virus is available, the virus content of the dodder is soon depleted, indicating that the virus is unable to maintain itself in dodder in the absence of an infected host plant. In most instances dodder appears normal when growing on diseased plants, but in some cases it shows distinct symptoms characteristic of curly-top. *C. californica* shows injury ranging from slight swelling and twisting of the stems to marked stem swelling, distortion, and darkening in certain areas, often with drops of phloem exudate on the stem surface. Some stems are dwarfed and the tips are killed; short lateral branches may appear forming a type of witch's broom. Sections of diseased areas show hypertrophy and cell collapse in the region of the phloem. None of these injuries has been noted on dodder growing on noninfected plants. It would appear, therefore, that under certain conditions curly-top virus is able to produce injury to plants that must be considered immune in the generally accepted connotation of this term.

Boron in relation to the culture of the peach tree. McLARTY, H. R., and C. G. WOODBRIDGE. When peach is grown on virgin soils in the Okanagan Valley of British Columbia it may suffer from boron deficiency. The outstanding characteristics are the failure of the buds to break in the spring, and an accompanying dying of twigs, branches, and sometimes of the entire tree. These symptoms may occur on trees that appeared to grow normally the previous season. Analyses of twigs from such trees indicate a boron content of 4–8 p.p.m. When too much boric acid is applied as a remedy, toxicity symptoms may occur. A slight excess produces small necrotic areas on the midrib or lateral veins of the leaves, while heavier applications may cause dying of

the terminal growth, yellowing of the foliage, severe cankering of the twigs and small branches, and malformation in the fruit. Chemical analyses of cankered twigs indicate a boron content of 116–125 p.p.m.

Alfalfa virus N. McWHORTER, FRANK P. This virus, here described as a necrotic strain of alfalfa mosaic virus, was isolated from alfalfa and peas in eastern Oregon and Washington. It was usually accompanied by yellow bean mosaic. The host range, including solanaceous hosts, is typical of alfalfa mosaic but the thermal inactivation point is 10° to 15° C. lower than those previously recorded for strains of that virus. It is placed as a strain of that virus because of its symptomatology in 8 families, 17 genera, and 19 species of plants. The designation N is given because the virus induces severe necroses that result in the death of many of its suscepts. Particularly distinctive is the death of many varieties of *Phaseolus vulgaris* L. that, following initial local lesions typical of alfalfa mosaic, develop dark necroses of the foliage and stems, wither, and die. Our field and laboratory records indicate that strains of alfalfa mosaic virus are of great economic importance in Oregon.

Pea enation mosaic, narcissus mosaic, and cancer. McWHORTER, FRANK P. The symptoms of pea enation mosaic in *Pisum sativum* L. and *Vicia faba* L. are production of translucent flecklike areas between or near veins, enations on the veins, and deformation of the pods. The translucent areas are usually composed of anaplastic cell masses produced by selective proliferation of veinlet vascular tissues often forming fanlike islands beyond the ends of veinlets. These masses are detrimental to the leaf and simulate a sarcoma. Likewise the deformation of the pods results from extreme hyperplasia and hypertrophy forming tissue that simulates both the form and abnormalities of neoplasms that lead to sarcomas in animals. The usual enations are overgrowths of epidermal cells; occasionally they are leaflike growths composed of normal cells. The roughening of narcissus foliage by narcissus mosaic is an enation due to extreme stimulation of epidermal cells. It is possible the legume and narcissus enation-producing viruses are closely related. The ability of pea enation virus to stimulate different primordial tissue (xylem parenchyma, for example) to form masses of anaplastic cells should receive consideration as a cancerlike phenomenon in plants where the cause is known to be a virus. Moreover, the malformations vary according to the tissue originally infected, a circumstance comparable to neoplasms in animals.

Weeds in relation to the results obtained from seed treatment in cereals and flax. MACHACEK, J. E. Treatment of diseased wheat, oats, and barley seed with New Improved Ceresan increased the yield by 13, 9, and 33 per cent respectively when the seed was sown in weed-free experimental plots. When sown in plots previously polluted with seed of Argentine rape (*Brassica napa* L.), used as an artificial weed, treatment increased the yield of these crops by 77, 62, and 83 per cent respectively. In flax, treatment decreased the yield by 13 per cent in clean plots but increased it by 13 per cent in weedy plots.

Soil fumigation for control of sweet potato black rot (Ceratostomella fimbriata). MEULI, LLOYD J., and ARTHUR W. SWEZEY. During the course of field experimentation on the control of root knot nematode (*Heterodera marioni*) and wireworm (*Limonius californicus*) with ethylene dibromide as a soil fumigant, it was observed that black rot disease was greatly reduced. Investigation over several years indicates that disease control is primarily indirect, and that at the dosages used the fumigant has little direct fungicidal action. The black rot organism, being principally a wound parasite, is controlled to a large extent by absence of wounds ordinarily caused by other soil pests. Material increases in yield and quality of tubers produced have resulted from soil fumigation. Maximum benefit in yield increase was obtained when optimum soil fertility was combined with soil fumigation.

Factors contributing to the infection of grapes by Botrytis cinerea Pers. NELSON, KLAYTON E. Botrytis rot has been a serious problem for the table grape growers of California in years when the grapes were still on the vines at the time of the first fall rains. In the present investigation using cold storage Emperor grapes it was ascertained that moisture was necessary to produce infection with conidia. At 3° C., 72 per cent infection occurred after 72–96 hr.; in the optimum range of 12° to 25°, over 75 per cent after 12–24 hr.; at 30°, nearly 58 per cent after 18–30 hr.; and at 35°, less than 15 per cent after 24 hr. The activity of other organisms obscured the symptoms of

Botrytis rot at 35°. A significantly greater amount of infection in compact than in loose bunches was found in only one of four trials. In the one case the average number of berries in the loose bunches was 54 per cent of that of the compact bunches. In the nonsignificant trials this number exceeded 60 per cent. Within the same bunch, berries with higher sugar percentage were significantly more susceptible to infection than those lower in sugar. Microscopic injuries and lenticels were of little importance as courts of infection; penetration commonly occurred through the unbroken skin.

Malachite green partially adsorbed on carbon-black and bentonite as sprays. NEWTON, WM. Solutions of malachite green and other highly soluble fungistatic substances may be converted into effective sprays by the addition of adsorbants. Malachite green was chosen as the active ingredient of the experimental sprays owing to evidence that it is 45 times as fungistatic as copper sulphate towards *Phytophthora erythroseptica.* A spray containing 0.05 per cent malachite green, 0.05 per cent carbon-black (norite), and 0.01 per cent Aerosol adhered well to potato foliage and the particles were well distributed. The norite reduced the concentration of the free dye from 0.05 per cent to 0.01 per cent. A spray in which the norite was replaced by the same weight of bentonite also appeared to adhere well, but the distribution on the foliage was less even. The bentonite reduced the free dye concentration from 0.05 per cent to 0.005 per cent. Both sprays containing the adsorbants appeared to create satisfactory barriers against *Phytophthora infestans* when applied to potato foliage as judged by the subsequent spraying of washed and unwashed excised leaves with inoculum, and both reduced the amount of powdery mildew on susceptible apple trees, but adequate information upon their efficiency as field sprays has not yet been obtained. Both adsorbants reduced the toxic effects of the malachite green solutions.

Vitamin K_5 as a preservative for fruits and a disinfectant for seeds. PRATT, ROBERTSON, JEAN DUFRENOY, and VIRGINIA L. PICKERING. Vitamin K_5, known chemically as 2 methyl-4-amino-1-naphthol hydrochloride, is an effective and potentially practical agent for use as a preservative for fruits and other economic products and as a disinfectant for seeds. It may be used in solution as a "dip" or as a dusting powder, either alone or dispersed in a dry, inert carrier. Vitamin K_5 has been used successfully in the laboratory to prevent spoiling of prunes, grapes, dates, various wines, and "soft" beverages and to prevent, rotting of seeds of peas, corn, cucumber, cotton, and mustard. K_5 is effective against different molds, yeasts, and bacteria in concentrations ranging from 0.001 to 0.03 per cent. Vitamin K_5 is effective also against several clinically important fungus pathogens of man.

Effect of time of inoculation with leafroll virus on the development of phloem necrosis in Russet Burbank potato tubers. RICH, AVERY E. Russet Burbank potatoes were grown under cloth cages at Pullman, Washington, in 1948. One month after planting, viruliferous peach aphids (*Myzus persicae*) were introduced into four cages containing four plants each. Every two weeks during the remainder of the summer a new set of cages was infested in the same manner. At maturity each hill was harvested separately and stored at about 60° F. for 2 months, and the tubers were then examined for phloem necrosis. The dates of infestation were July 4 and 18, and Aug. 1, 14, and 28. The percentages of tubers with phloem necrosis were 79, 60, 69, 67, and 49 respectively. The noninfested controls exhibited no phloem discoloration. The average depth of penetration of the necrosis into the tubers from the stem end varied little, ranging from 1.7 cm. for the lot which was infested July 4 to 2.8 cm. for the lot which was infested Aug. 1. The plants which were infested early in the season produced smaller tubers than the others with a corresponding reduction in yield. All of the tubers were indexed in the greenhouse. The tubers from the inoculated plants produced plants with typical leafroll symptoms while the tubers from the noninfested plants produced apparently healthy plants.

Effect of various methods of killing potato vines on rate of defoliation and internal discoloration of tubers. RICH, AVERY E. In 1947 an attempt was made to find a potato vine killer that would not injure the tubers. Sodium arsenite, dinitro-o-sec. butyl phenol, dinitro-o-sec. amyl phenol, and copper sulfate were applied to nearly mature potatoes at Pullman. All materials except the last gave a satisfactory kill within 3 days with no serious injury to the tubers, probably due to their stage of maturity. In 1948, pentachlorophenol, kerosene, cyanamid aero-defoliant, and sulfate of ammonia were also tried. All materials except kerosene and cyanamid killed the vines rather rapidly. However,

some of them caused considerable xylem discoloration. The phenol-containing compounds were the worst, with from 47 per cent to 98 per cent of the tubers showing serious injury. Sodium arsenite, copper sulfate, and sulfate of ammonia produced a slight amount of discoloration, but less than mechanical methods or frost. Sulfate of ammonia (2 lb. per gal.) looks very promising because, in addition to giving a satisfactory kill with a minimum amount of tuber injury, it is nonpoisonous, is a nitrogen carrier, is not a fire hazard and is readily available. In a large field trial it killed more rapidly than copper sulfate and caused less xylem discoloration than did the frost which followed.

Downy mildew immunity in spinach. SMITH, PAUL G. Of 17 commercial varieties of spinach tested for resistance to downy mildew, all were found to be highly susceptible. Attempts to select resistant individuals from more than 50,000 Viroflay and Prickly Winter inoculated were unsuccessful, indicating little chance of obtaining resistance within these varieties by selection. Nine Bureau of Plant Exploration and Importation lots were tested. About 50 per cent of the plants of one, P. I. 140,467, were found to be immune. Tests on the F_1 and first backcross populations in crosses with susceptible commercial varieties indicate control of immunity by a single dominant gene.

Cross inoculations with the vascular Fusaria of stock, cabbage, and radish. SNYDER, WILLIAM C. Although it was found occasionally possible to obtain cross infections between the vascular Fusaria of stock, cabbage, and radish and their hosts, each pathogen consistently showed a highly selective specificity for its own host. The results would seem to warrant the distinction of these three vascular pathogens as separate biological forms of *Fusarium oxysporum.*

The strawberry virus complex. THOMAS, HAROLD E. Using *Fragaria vesca* var. *californica* as an indicator plant the viruses found in limited collections of strawberry plants, particularly the Marshall variety, from Pacific Coast and Eastern United States, have been indexed. Healthy appearing Marshalls from the Pacific Coast have indexed out a set of symptoms termed "mottle." These are characterized by small and distorted leaves with some downward cupping, mild leaf puckering, and moderate yellowing with islands of green to give a mottled appearance. Initial shock may cause killing of young central leaves. Crown necrosis is often observed. Indicator plants become dwarfed and stunted. Mottle appears to be a component of the yellows (Xanthosis) virus. Marshall plants from the Eastern United States exhibiting very mild marginal yellowing indexed out an entirely different set of symptoms. These are characterized by a twisting, drooping, and curling of the young central leaves resulting in a twisting of the crown with stunting. Mild yellowing may occur. Because of curling and drooping downward of the young leaves the term "droop" was given this set of symptoms. There is no certainty that this virus entity has any relation to Pacific Coast Xanthosis. Symptoms of Xanthosis in indicator plants were more severe than either mottle or droop.

Raspberry yellow rust control. VAUGHAN, EDWARD K. Fungicidal sprays gave satisfactory control of yellow rust of red raspberry (*Phragmidium rubiidaei*) in field tests in Oregon during the seasons of 1948 and 1949. A single application was made when the unfolding buds had pushed out approximately 1 in. and were beginning to show green. The number of aecia on the foliage of bearing canes was only 1 to 10 per cent of that on unsprayed plants. At the harvest season plants sprayed with Lime-Sulphur or Phygon-XL had the least amount of yellow rust. Elgetol, Cop-O-Zink, and Fermate gave almost as good control. All sprayed plots had significantly less yellow rust than the unsprayed checks.

Inoculation technique, incubation period, and early symptoms of the quick decline disease of citrus. WALLACE, J. M. The destruction of phloem tissues of trees of sweet orange on sour orange root-stocks affected by quick decline induces a girdling effect and resultant symptoms are those of a girdled tree. Trees 1 to 2 years of age usually show no marked top symptoms until 12 to 15 months after infection. Young trees flower and fruit prematurely; this is a useful index of infection. These symptoms can sometimes be observed within 8 to 10 months after infection. A still shorter test is needed, particularly for use in the search for the insect vector or vectors of the virus. Young trees inoculated in early September and cut back very severely produced a flush of new leaves and then went into winter dormancy. The following January inoculated trees showed small, off-color leaves, an absence of stored starch in roots, and slight rotting of the feeder roots. Healthy control trees similarly pruned were normal. Because of the

vigorous growth of young trees during the spring and summer the inoculation technique described herein may not shorten the time required for symptom expression, but it is hoped that certain modifications of these methods may provide a short time test for quick decline transmission.

Vertical distribution of Verticillium albo-atrum in some California soils. WILHELM, STEPHEN. Quantitative information on the vertical distribution of *Verticillium albo-atrum* Rke. et Bert. was obtained for a variety of soils by a standardized procedure using infection of the Bonny Best tomato variety as a measure of the intensity of infestation. Of 20 depth samples from Verticillium-infested fields representing mainly cotton, potato, or tomato land, Verticillium was found as follows: 19 instances in the 0–6-in. depth, 19 instances in the 6–12-in. depth, 10 instances in the 12–18-in. depth, 10 instances in the 18–24-in. depth, 8 instances in the 24–30-in. depth, and 3 instances in the 30–36-in. depth. The 0–6-in. and 6–12-in. depths were found on the whole to contain 3 to 4 times the degree of infestation as the deeper soil layers. The vertical distribution of *V. albo-atrum* is believed to be governed by factors not related to depth of root penetration, soil type, or climatic environment.

Effect of temperature on the fungicidal activity of sulphur. YARWOOD, C. E. Disks of 3.5-mm. diameter cut from living bean leaves, sprayed 1 hr. previously on both surfaces with 10 per cent lime sulphur, were placed on the surface of mildew-inoculated detached bean leaves in Petri dishes on 8 per cent sucrose. The inoculated leaves with disks bearing the dried deposit of lime sulphur were placed at a range of test temperatures for a range of test intervals. The lime sulphur disks were then removed and the leaves were placed at 24° C. The growth of mildew (*Erysiphe polygoni*) was inhibited in circular zones beyond the position of the lime surphur disks. The time to inhibit a zone 1 cm. in diameter was about 70 hr. at 7° C., 30 hr. at 13°, 5 hr. at 25°, 2 hr. at 31°, 45 min. at 37°, and 20 min. at 43°. For a 10° increase in temperature, the fungicidal activity of sulphur was increased about 4.5 times, while the vapor pressure of sulphur (Menzies, Int. Critical Tables 3: 201, 1928) was increased only 2.2 times. With bean rust (*Uromyces phaseoli*) the relative activity due to temperature of hydrogen sulphide in therapy trials was about the same as for lime sulphur against bean powdery mildew.

Fungicidal action of volatile soil fumigants. ZENTMYER, G. A., and J. B. KENDRICK, JR. In laboratory experiments to determine direct fungicidal action of soil fumigants, materials tested against *Fusarium solani* f. *phaseoli*, *Phytophthora cinnamomi*, *Rhizoctonia solani*, and *Thielariopsis basicola* were: 1,3-dichloropropene, 1,2-dichloropropane, Dowfume N (mixture of 1,3-dichloropropene and 1,2-dichloropropane), ethylene oxide, ethylene dibromide, carbon disulfide, and chloropicrin. The materials were tested under sterile conditions in closed jars, with and without soil; inoculum was removed and cultured after 24 hr. incubation at 24° C. Three dosages were used for each material. Chloropicrin, ethylene oxide, 1,3-dichloropropene, and Dowfume N were the most effective fungicides. Chloropicrin killed all four fungi at a dosage of 1.9 ml/ft³; ethylene oxide killed all but Fusarium at 1.4 ml/ft³; 1,3-dichloropropene killed all but Fusarium at 2.1 ml/ft³; Dowfume N killed all but Rhizoctonia at 2.1 ml/ft³. Ethylene dibromide killed Phytophthora at 2.1 ml/ft³; 2 to 4 times this dosage was required to kill the other fungi. Carbon disulfide was fungicidal against Rhizoctonia at 4.2 ml/ft³; 8.4 ml/ft³ was lethal to the other fungi. 1,2-Dichloropropane was ineffective as a fungicide even at 8.4 ml/ft³. Considering all materials, *P. cinnamomi* was the least resistant to the fumigants, followed by *T. basicola*, *R. solani*, and *F. solani* f. *phaseoli* in that order.

HOWARD SAMUEL FAWCETT, 1877–1948

L. J. Klotz and Eubanks Carsner

Howard Samuel Fawcett, Professor Emeritus of Plant Pathology of the University of California Citrus Experiment Station, died at Riverside, California, December 12, 1948. Botanical science thus loses a world authority. Professor Fawcett was an outstanding investigator of citrus diseases, his chosen field.

He was born on a farm near Salem, Ohio, on April 12, 1877. His early education was at Salem where he completed the curriculum of the local high school. Then after a year's attendance at Westtown School, a Friends' preparatory school at Westtown, Pennsylvania, where he studied botany under Dr. Henry S. Conard, he was graduated in 1899. After a year (1900–1901) of teaching science in a preparatory school at Le Grande, Iowa, he went to Iowa State College where, working his way as teaching assistant to Dr. L. H. Pammel, he completed the science course in 1905, earning the B. S. degree. He filled the position of Assistant in Botany and Horticulture at the University of Florida during the year following his graduation and became Assistant Plant Pathologist of that institution in 1907 and Plant Pathologist in 1908. In 1908 he earned his M. S. degree there. During his seven years in Florida he made important contributions on fungus diseases of citrus insects, on scaly bark and gummosis diseases of citrus trees, and on stem-end rot of citrus fruits. While at the University of Florida he was associated with H. J. Webber, W. T. Swingle, and P. H. Rolfs, pioneers in citrus investigation.

In 1912 he accepted the position of Plant Pathologist with the California State Commission of Horticulture, his immediate objective being to investigate the destructive gummosis or footrot disease that was ravaging the citrus groves of that State. After a year with the Commission he joined the staff of the University of California as Associate Professor of Plant Pathology, continuing his work on gummosis. During the next three years he isolated the causal fungi, proved their relationship to the disease, and developed successful methods of treatment—results of great importance to the industry.

The years 1916 to 1918 were spent on leave at Johns Hopkins University where under Dr. Burton E. Livingston he investigated the temperature relations of certain fungi parasitic on citrus trees, and developed apparatus for temperature control which has proved very useful in botanical investigations. He received the Ph.D. degree at that institution in 1918.

As Professor of Plant Pathology in the University of California and Plant Pathologist in the Agricultural Experiment Station, Dr. Fawcett served the University and State of California from 1918 to 1947, when he became Professor Emeritus. He turned over the administrative duties of

865

the Division of Plant Pathology in 1946 to his successor, to devote himself to research on diseases of citrus. He continued intensively active and productive until his death in December, 1948. Upwards of 300 articles were contributed in scientific and industrial journals. He introduced the scraping treatment for citrus scaly bark (psorosis) in 1922, eleven years before he demonstrated the virus nature of that malady. His discovery of the causes of gummosis and psorosis and his development of practical means for their control have been of inestimable value in the successful production

HOWARD SAMUEL FAWCETT

1877–1948

of citrus in California and other areas. During the last few years he played the leading rôle in the investigation of the cause of quick decline of orange trees, demonstrating its virus nature. In 1940 he proposed "a simple, easily applied pro tem manner of naming viruses" in which the stem "vir" was added to the Latin genitive of the genus of the host in which the virus was first discovered and recognized, dropping the final consonants of this genitive. Quick decline and other virus and virus-like diseases of citrus, including stubborn disease of sweet orange, wood pocket of lemon, exocortis of trifoliate orange, and the various forms of psorosis were his major research interests in recent years. The great importance of his work to the citrus

industry is recognized by scientists and by growers and processors of citrus fruits throughout the world.

In the first edition of the book "Citrus Diseases and Their Control," published in 1926, he collected his own contributions and the world's knowledge of citrus diseases. Dr. H. A. Lee, who was junior author of this first edition, wrote certain sections on the diseases in the Orient.

Dr. Fawcett studied citrus and date diseases in the Mediterranean countries of Europe, North Africa, and Palestine during 1929 and 1930, serving as a Collaborator of the United States Department of Agriculture. He investigated citrus troubles in Brazil and Argentina in 1936 and 1937. His findings in these travels were incorporated in technical papers and in the revision of his book, which has become the standard reference work and textbook in this field.

The second edition of his book was published in 1936 and reprinted in 1946. Dr. Fawcett took up the responsibility and authorship of this edition since Dr. H A. Lee had given up research in the field of citrus and requested that his name be omitted. In 1948, he contributed to Volume II of "The Citrus Industry" a chapter on control of citrus insects by fungi and bacteria, and, as senior author, the chapter on "Diseases and Their Control." His textbook has been translated into Hebrew and may also be printed in Spanish and Portuguese editions. With L. J. Klotz he authored in two editions, 1941 and 1948, a "Color Handbook of Citrus Diseases" which provides citrus growers, packing house men, horticultural inspectors, and extension workers with a ready means of identifying citrus diseases together with the essential information on control.

Professor Fawcett was a charter member of The American Phytopathological Society. He was vice-president of that society in 1929 and president in 1930. He was a member of Phi Beta Kappa, Sigma Xi, the Botanical Society of America, the Mycological Society of America, Societa Internazionale di Microbiologia (Milano), and Fellow of the American Association for the Advancement of Science. His name was starred in the Fifth Edition of "American Men of Science" (1933), which means his selection then as one of 250 leading American scientists and one of 25 leading botanists. In 1940 Dr. Fawcett was chosen to give the annual Faculty Research Lecture at the University of California at Los Angeles. Some of his most outstanding work is described most fascinatingly in that lecture, "Adventures in the Plant Disease World."

Dr. Fawcett's energy and enthusiasm in his research activities, his good fellowship, patience, kindness, and fairness in all relationships with his friends and fellow investigators will always be an inspiration and guide to those who have been so fortunate as to have known him and to have been associated with him.

He was a devoted, birthright member of the Society of Friends and a leading figure in the establishment and support of the Riverside Friends

Meeting. He worked quietly and persistently for the advancement of peace and goodwill in all human relationships and for relief of suffering wherever it occurred. He served in the famine-stricken area of southeastern Russia in 1922–1923, during a sabbatical leave, as a member of a mission sent by the American Friends Service Committee. The constructive program of ministration to the stricken people there, and the example of the members of the mission, laid a foundation of goodwill among the Russian people of that region.

Surviving Professor Fawcett are his widow, T. Helen Tostenson Fawcett of Riverside, California; a daughter, Rosamond Fawcett Leuty of Whittier, California; two brothers, Luther T. Fawcett of Youngstown, Ohio, and Ralph F. Fawcett of Asheville, North Carolina; and a sister, Esther S. Stanton of Westtown, Pennsylvania.

CABBAGE SEED TREATMENT[1]

GLENN A. HUBER[2] AND CHARLES J. GOULD[3]

INTRODUCTION

Several hundred acres of cabbage are grown for seed each year in western Washington. For such a purpose the stock seed is planted in beds during June and July and the plants are transplanted to the fields during late summer and early fall.

Fortunately, certain diseases of cabbage such as blackleg, black rot, and yellows have not yet become established in western Washington, possibly because of the cool, mild climate in this area. Other diseases, however, sometimes cause damage to the seed crop, especially in years when wet weather prevails during the spring and early summer months. One of these diseases is the mycosphaerella ringspot, commonly called "black blight," caused by *Mycosphaerella brassicicola* (Fr.) Lindau.

A survey of several cabbage plant beds in Skagit County, Washington, during 1941 and 1942 revealed the presence of black blight lesions on the lower leaves of certain plants. These infections may have come from wind-borne spores or from infected seed. The possibility of plant bed infection arising from the use of infected seed was therefore investigated.

Mature seed pods bearing large lesions of black blight were collected from severely infected cabbage plants. Microscopic examinations showed that the fungus had penetrated the seed coats and in a number of cases had produced lesions on the underlying cotyledons. *Mycosphaerella brassicicola* was isolated from a number of seeds so infected. Weimer[4] had also found that the fungus was capable of growing through the seed pods into the seed and, in a few cases, into the cotyledons. However, he did not find cotyledon infection in the plant bed.

Seed taken from beneath large lesions on the pods were planted in the greenhouse. Many of the seedlings emerged with definite lesions on the cotyledons. In similar field experiments occasional lesions were found in isolated plant beds on seedlings grown from seed selected the previous year from heavily infected pods. Greenhouse trials did not indicate that the seed-borne organism was capable of causing damping-off of seedlings.

The relatively small amount of infection which may result from infected seed appears to be of little or no consequence where cabbage is

grown for market or home use. However, the use of infected seed as stock seed for furthering seed production is of considerable consequence, since only an occasional infection in a field in the fall may result in a build-up of the disease to damaging amounts by harvest time the following year.

The importance of using as stock seed only "clean" seed, or seed treated so as to kill the fungus on and in the seed or on debris carried with the seed, cannot be too strongly emphasized since the use of infected seed is an important factor not only in introducing the disease into the plant bed but also in introducing it into new areas.

A hot water treatment of seeds has been recommended for controlling such cabbage seed-borne diseases as Alternaria blight, blackleg, and black rot. Haskell and Doolittle[5] summarized the data on temperature and duration of hot water treatments as presented by various investigators and recommended hot water at a temperature of 122° F. (50° C.) for 25 min. for cabbage seed. Walker[6] pointed out that the hot water treatment must be used with great care, since it is likely to reduce germination somewhat. Old seeds were more susceptible to injury. Weimer[4] suggested treating cabbage for *Mycosphaerella brassicicola* by immersing the seed in water for 10 min. at 55° C. or for 30 min. at 50° C. which is the same treatment suggested by Walker[7] for *Alternaria brassicae*. Since Weimer found that *M. brassicicola* was "considerably more sensitive" to heat than *A. brassicae* it seemed logical that a lower temperature could be used in treating cabbage seed for eliminating *M. brassicicola*.

In order to secure information pertaining to treatment of cabbage seed as a supplementary control measure for mycosphaerella ring spot (black blight), a series of experiments were made in 1941–1943, inclusive.

MATERIALS AND METHODS

The procedure used in treating the seed in hot water was as follows: The seed was weighed out in 5-gm. lots, each being placed in a small cheesecloth bag, leaving only enough space in each bag for swelling of the seed. The bags of seed were placed in the top compartment of a large double boiler where the water had been raised to slightly above the desired temperature. The addition of the seed lowered the temperature of the water to the desired temperature at which it was maintained throughout the treatment. The bags of seed were agitated frequently. Chemical treatment was executed by placing the seed with the required amount of chemical in 250-cc. flasks and vigorously shaking for 3 min. The chemicals used were: the mercury compounds (calomel, corrosive sublimate, Barbak-C, and Semesan), tetramethyl thiuramdisulphide (Arasan and Tersan),

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[5] Haskell, R. J., and S. P. Doolittle. Vegetable seed treatments. U. S. Dept. Agr., Farmers' Bul. 1862. 1940. (Revised 1942.)

[6] Walker, J. C. Diseases of cabbage and related plants. U. S. Dept. Agr. Farmers' Bul. 1439. 1934.

[7] Walker, J. C. Seed treatment and rainfall in relation to the control of cabbage blackleg. U. S. Dept. Agr. Bul. 1029. 1922.

tetrachloro parabenzoquinone (Spergon), a carbamate (Fermate), and zinc oxide.

All treated and nontreated samples were replicated four or more times. Effectiveness against fungi was determined by plating 100 seeds of each replicate of treated and nontreated seed on potato-dextrose agar and incubating for 8 days at room temperature. Germination of seed was determined by placing 100 seeds of each replicate in the seed-germinator, and recording percentage germination after ten days. Percentage stand

TABLE 1.—*The effect of various seed treatments on germination of seed in Petri dishes and on numbers of fungi carried on and in cabbage seed, variety Marion Market*

Treatment	Percentage germination[a]	Percentage of seeds producing fungus colonies
Nontreated check	84.7	21.3
Arasan, 1 per cent	98.0	1.3
Barbak-C, 2 per cent	89.3	3.7
Calomel, as much as seed would carry	95.0	1.7
Corrosive sublimate, 1–1000, 20 min. dip	89.3	3.0
Semesan, 0.4 per cent	82.0	1.3
Spergon, 2 per cent	91.3	3.7
Hot water, 45° C., 20 min.	97.7	0.0
Hot water, 50° C., 20 min.	91.0	0.0
Hot water, 50° C., 20 min.; seed dried, treated with Arasan, 1 per cent	87.0	0.0
Hot water, 50° C., 20 min.; seed dried, treated with Barbak C, 2 per cent	92.0	0.0
Hot water, 50° C., 20 min.; seed dried, treated with Calomel, as much as seed would carry	86.7	0.0
Hot water, 50° C., 20 min.; seed dried, treated with Semesan, 0.4 per cent	19.3	0.0
Hot water, 50° C., 20 min.; seed dried, treated with Spergon, 2 per cent	86.3	0.0
Hot water, 50° C., 20 min.; seed dried, treated with zinc oxide, 2 per cent	90.0	0.0
F value (5.39 needed at 1 per cent level for significance)	29.4	47.0
Difference required for significance (5 per cent)	9.3	2.2
Do (1 per cent)	12.5	3.0

[a] Percentage is the same as the mean in these tests, since 100 seeds were used per replicate.

was determined by planting 100 seeds of each replicate in the greenhouse in sterilized Puyallup sandy loam soil.

RESULTS

Table 1 presents results of an experiment to determine the effect of various seed treatments, including treatments previously recommended, on germination and on fungi carried on and in the seed. A relatively high germination was obtained in all sets with the exception of the one soaked in hot water, dried, and treated with Semesan.

Since treatment of seeds in hot water at 45° C. for 20 min. gave an improvement in seed germination and killed all the fungi carried on and

in the seed, it was deemed advisable to determine the effect of hot water treatment at different temperatures and for different periods of time on germination of the seed and on fungi carried on and in the seed. Table 2 gives data on such treatments.

Treating the seed with hot water at 45° C. for 20 min. did not materially reduce germination or stand but did kill all the fungi carried on and in the seed. Seed selected from pods having heavy ring-spot infection was treated with hot water at 45° C. for 20 min. and was immediately plated on potato-dextrose agar. No fungus colonies developed from the treated seed, while several fungi, including *Mycosphaerella brassicicola*, developed from the nontreated seed.

TABLE 2.—*The effect of hot water treatment on germination, on stand, and on the number of fungi carried on and in cabbage seed, Marion Market variety*

Temperature (degrees C.)	Period of treatment	Germination	Stand	Seeds producing fungus colonies
	Minutes	Per cent	Per cent	Per cent
16	10	89.8	78.5	8.2
	20	88.8	80.8	9.5
	30	89.2	80.5	9.0
40	10	91.2	80.5	6.0
	20	89.5	81.2	5.5
	30	89.0	81.2	2.2
45	10	90.2	79.2	0.5
	20	89.5	79.2	0.0
	30	87.0	77.2	0.0
50	10	80.5	76.8	0.0
	20	80.5	58.0	0.0
	30	73.2	57.0	0.0
55	10	74.2	49.0	0.0
	20	47.8	32.8	0.0
	30	32.8	10.2	0.0
F value (2.5 needed at 1 per cent level for significance)		42.8	105.0	18.2
Difference required for significance (5 per cent)		7.5	6.0	2.5
Do (1 per cent)		10.0	8.0	3.4

From the results obtained in this and in previous experiments, hot water at 45° C. for 20 min. was selected as the standard hot water treatment for further experimentation.

RELATION OF HOT WATER TREATMENT TO AGE OF SEED

The relation of hot water treatment to age of seed and to different varieties was investigated in another experiment. Treatment of 1-year-old seed of four varieties (Steins' Flat Dutch, Early Round Dutch, Copenhagen Market, and Red Danish) generally increased germination up to 2.75 per cent. There was no strong indication of differences between these varieties, in which germination was over 94 per cent. Three-year-

old seed of other varieties, however, suffered from treatment, the germination dropping from 86.5 to 62.2 per cent in Marion Market, 77.2 to 69.5 per cent in All Head, and 50.2 to 47.0 per cent in Danish Ballhead. Seed of three other varieties, the ages of which were unknown, were also injured by treatment and germination counts were lowered as follows: Glory of Enk-

TABLE 3.—*The effect of hot water at 45° C. for 20 min., followed by various chemical treatments, on seed germination and on stand of cabbage plants, Marion Market variety*

Chemicals	Percentage germination					Percentage stand				
	Chemical treatment only	Hot water treatment followed by chemical treatment when seeds were:				Chemical treatment only	Hot water treatment followed by chemical treatment when seeds were:			
		Wet	Partly dry	Dry	Av.		Wet	Partly dry	Dry	Av.
Nontreated check	80.0	88.2	89.0	87.0	86.0	50.2	50.0	42.6	44.6	46.8
Semesan, 0.4 percent	77.0	88.6	79.6	89.0	83.6	80.2	79.4	68.8	70.4	74.7
Zinc oxide, 2 per cent	86.2	86.8	80.4	94.0	86.8	79.6	71.4	79.6	72.0	75.7
Calomel, 5 per cent	82.2	86.2	82.6	93.2	86.0	71.2	69.2	73.4	71.0	71.2
Tersan, 1 per cent	80.0	84.2	75.4	87.2	81.7	83.8	84.8	85.2	85.0	84.7
Arasan, 1 per cent	86.0	89.0	85.8	90.6	87.8	83.0	82.0	81.6	83.0	82.4
Fermate, 1 per cent	85.6	89.0	88.4	93.4	89.1	74.2	55.0	71.8	69.8	67.7
Spergon, 2 per cent	84.2	84.0	76.8	93.2	84.6	71.4	74.0	77.6	81.2	76.0
Av.	82.6	87.0	82.2	91.0	85.7	74.2	70.7	72.6	72.1	72.4

	F calculated	F needed 5 per cent	1 per cent	LMD 5 per cent	1 per cent
Germination: between chemical treatment and hot water treatment	12.4	1.5	1.8	3.9	5.2
Stand: between chemical treatment and hot water treatment	29.2	1.5	1.8	6.1	8.1
Between hot water and no hot water treatments	3.4	2.7	3.9	2.2	2.9
Between chemical treatments	114.9	2.1	2.8	3.1	4.0
Treatment interaction	4.5	1.6	2.0		

huizen, from 68.8 to 59.0 per cent; Premium Flat Dutch, from 43.0 to 11.8 per cent; and Copenhagen Market, from 32.2 to 18.2 per cent.

CHEMICAL TREATMENT FOLLOWING HOT WATER TREATMENT

The following experiment was set up to determine the effect of various chemical treatments following the hot water treatment on germination of the seed in Petri dishes and on stand of plants from seed planted in non-sterilized soil. The objective of the double treatment was to kill seed-

borne organisms with the hot water and to protect the seed from soil-borne fungi with the chemical treatment. Five replications were made with each treatment. In the first set the chemicals were applied while the seed was still wet from the hot water treatment; in the second set they were applied 12 hr. later when the seed was partially dry; and in the third set chemical treatment was delayed until the seed had been dried for 60 hr. in the laboratory.

With few exceptions the percentage germination shown in table 3 was greater than the percentage stand, the greatest difference being in the nontreated check and in lots treated only with hot water. These results show that hot water treatment of cabbage seeds should be followed with a chemical treatment before seed are planted in soil. Tetramethyl thiuramdisulphide, either in the form of Tersan or Arasan, consistently improved the

TABLE 4.—*The effect of soaking in hot water at 45° C. for 20 min., followed 60 hr. later with 1 per cent by weight of Arasan, on germination of seed and on stand of several varieties of cabbage*

Variety	Germination			Stand		
	Nontreated	Treated	F[a] value	Nontreated	Treated	F[b] value
Charles Wakefield	98.4	96.0	6.5	61.2	91.8	61.8
Copenhagen Market	88.4	92.8	6.5	24.2	87.4	244.4
Early Round Dutch	95.0	92.4	N.S.	60.2	90.4	359.1
Jersey Wakefield	90.8	92.8	N.S.	27.4	81.6	206.3
Marion Market	27.8	72.6	254.1	6.0	46.4	3400.3
Penna. State Ballhead	55.8	87.4	109.7	40.6	68.8	1242.5
Red Danish	80.8	92.6	81.9	42.4	74.0	84.3
Steins' Flat Dutch	82.8	71.8	23.8	41.2	67.0	28.8

[a] F needed for significance is 5.3 (5 per cent) and 11.3 (1 per cent).

[b] F needed for significance is 7.7 (5 per cent) and 21.2 (1 per cent).

stand of plants over the other treatments. The mean stand difference between treatment with this and other materials was highly significant. The difference in stand was also highly significant between seed treated only with the chemicals and seed treated with chemicals immediately after hot water treatment In the latter part of the test, zinc oxide and Fermate were responsible for the greatest reduction. The differences between the various treatments of dry and wet seeds with Tersan and Arasan were negligible. The analysis of the data for stand indicated that there was also a highly significant interaction between chemical treatment and hot water treatment. This is especially evident if we compare results for the seeds treated with Semesan or Spergon and results for the seed treated with these materials 60 hr. after being treated with hot water. Semesan gave best results on seed not treated with hot water, but the reverse was true of Spergon.

HOT WATER TREATMENT OF DIFFERENT VARIETIES

In another experiment the seed of several varieties of cabbage was treated 8 months after harvest with hot water at 45° C. for 20 min. After being dried at room temperature for 60 hr., the seed was treated with 1 per cent by weight of Arasan. The results are given in table 4.

As in the preceding experiment, the percentage stand was less than the percentage germination. However, the percentage stand of the treated seed in every variety was significantly higher than the percentage stand of the nontreated seed.

SUMMARY

1. Treatment with hot water at 45° C. for 20 min. caused little or no injury to vigorously germinating cabbage seed and was effective in destroying fungi, including *Mycosphaerella brassicicola*, carried on and in the seed.

2 Three-year-old seed of certain varieties was definitely injured by the hot water treatment.

3. The hot water seed treatment, followed by various chemical treatments, resulted in a highly significant increase in stands of cabbage plants over the nontreated check.

4. The hot water treatment (45° C. for 20 min.) followed by 1 per cent by weight of Tersan or 1 per cent of Arasan 60 hr. after hot water treatment proved the most effective double treatment.

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MODE OF INFECTION OF THE SWEET-POTATO WILT FUSARIUM[1]

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The Fusarium wilt disease of sweet potato (*Ipomoea batatas* Poir.), known also as stem rot, is caused by the soil-inhabiting vascular pathogen, *Fusarium oxysporum* f. *batatas* (Wr.) Snyd. *et* Hans. Invasion of the stem by this fungus produces a brown discoloration of the affected xylem, a yellowing of the leaves, and eventually the death of the shoot. The investigation reported here on the mode of infection was undertaken with the hope that a basis might be provided for a sound approach to control.

REVIEW OF LITERATURE

Infection of sprouts from diseased mother potatoes by way of the vascular connection between the two has been shown by Harter and Field (6), Harter and Weimer (7), and Poole (14). These authors, however, agree that the incidence of infection from this source is considerably less than that found in the crop, and suggest that most of the infection of the crop may come from the field soil. Furthermore, Harter and Field (6) cite experimental evidence that only a low percentage of the sprouts formed by diseased potatoes are diseased.

Infection of sprouts via the basal wound formed when the sprout is drawn from the mother potato, and via other wounds, has been claimed by Gregory (4), Harter and Weimer (7), and Poole and Woodside (16). Watanabe (21) obtained mild infection when the inoculum was inserted into a vine, but no infection resulted from placing the inoculum on the surface of the vine.

Infection of the sprout by way of its roots has been claimed by Daines (2), Harter (5), and Taubenhaus (20), and listed as rare by Poole and Woodside (16). The necessity of wounds for infection was not specified by these authors, but Daines (2) advises against fertilizer injury to the roots, and Poole and Schmidt (15) claim that, by providing wounds on the roots, nematodes aid the entry of the pathogen.

Much of the rest of the literature on this disease is concerned primarily with control practices which only indirectly imply the mode of infection. The three categories of control practices are hill selection, seed dipping, and sprout dipping.

Hill selection is the practice of saving seed potatoes from healthy plants, and implies that the sprouts become infected from their mother potatoes

and that potatoes are not infected from the tail or root-tip end. Some of the advocates of hill selection are Daines (2), Gregory (4), Harter (5), Manns (11), and Poole (13).

The immersion of seed potatoes in some fungicidal solution prior to bedding implies that healthy potatoes may be infected from the hotbed soil, resulting in the production of diseased sprouts. Layton (10) reported that seed dipping increased the percentage of healthy plants, but his data show this increase to be small. Other advocates of seed dipping include Elmer (3), Gregory (4), and Harter (5). It is evident that the fungicide deposited on the surface of the potato will not cover the wounds formed later when the first crop of sprouts is pulled. Harter and Whitney (8) contaminated healthy uninjured seed potatoes with a spore suspension of the pathogen and found a low percentage of infection in the first crop of sprouts, and a higher percentage of infection in the subsequently formed sprouts.

The immersion of the roots and bases of sprouts in a fungicide prior to planting implies that the sprouts become infected from the field soil and that the fungicide will protect the sprout from such infection. Some of the advocates of sprout dipping are Elmer (3), Layton (10), and Manns and Heuberger (12). Porter and Parris (17) presented evidence that of five chemicals commonly used in sprout dipping, none showed any statistically significant effect in reducing the incidence of Fusarium wilt.

MATERIALS AND METHODS

Seed potatoes of the Porto Rico Red variety were obtained from growers in the San Joaquin Valley and bedded in an electrically heated, formalin-disinfected hotbed in the usual manner. When sprouts 6 to 8 in. tall were obtained, tip cuttings were made with a flamed razor, the cut being made about 1 in. below a node on the aerial part of the stem. The discarded part of each sprout was then examined to insure that only originally healthy sprouts were used. Vascular discoloration was relied upon to reveal the presence of the disease, since no instance was ever found where the pathogen was present in the absence of discoloration.

Freshly cut sprouts could be inoculated by dipping the fresh basal wound into a spore suspension of the pathogen. The sprout cuttings were set in steamed sand and placed in a warm chamber kept moist by a fine spray of water. After 4 to 6 days, roots were usually developed, and the basal wound of healthy cuttings was usually healed by the formation of a callus. After the plants were gently separated from the sand, healed plants could be inoculated by dipping the entire root system and stem base in a spore suspension. All plants, whether inoculated or not, were immediately transplanted to moist, steamed soil in 5-in. pots and set in the greenhouse. No manual firming of the soil was practiced lest breaking of roots result, but the soil was settled about the roots by watering the pots.

At the termination of each experiment the plants were collected with as much of the root system as possible and were washed free of adhering soil. A plant was considered infected if it had the vascular discoloration typical of the disease. The roots and freehand transverse sections of the stem were examined under a dissecting microscope. This method was more accurate for stems than that of peeling away the cortex. Freehand, transverse sections of stems and roots, surface-disinfected with a mercuric chloride solution (1:1000 in 10 per cent ethyl alcohol), were cultured on acidified potato-dextrose agar. The results of these cultures corroborated the diagnoses based on vascular discoloration, and in representative cases the isolates obtained were tested on healthy cuttings and produced the Fusarium wilt disease.

The cultures of *Fusarium* used in these experiments were all originally isolated from infected sweet potatoes and were maintained by single-spore transfers to potato-dextrose agar in culture tubes.

Spore suspensions were made by adding water to a 2-week-old culture, rubbing the mycelial mat with a glass rod to aid spore liberation, and then decanting the spore-laden water. A spore suspension of approximately 400 cc. was made from one tube culture.

EXPERIMENTAL RESULTS

Identification of the Pathogen

Twenty-six single-spore cultures of *Fusarium oxysporum* isolated from sweet potato were examined and it was found they could be separated into three distinct groups by their cultural characteristics (Fig. 1). Some slight differences were noted between the members of each group, indicating the presence of several clones. Mutants were segregated by single-spore transfers from old cultures of each group, but in no case did a mutant of one group show the characteristics of one of the other groups.

All of the cultures were tested on freshly cut sprouts to ascertain which were of the wilt pathogen. At first the plants were examined after only 1 week, but it was soon found that 2 weeks were preferable, in order to give sufficient time for the pathogen to grow upward in the stem to the ground level where it could be reisolated readily. In addition to the vascular discoloration and other previously described symptoms, all cultures of the pathogen caused a brown rot of the basal wound. The wilt pathogen was the only one that could be isolated from such lesions.

Eleven of the 26 cultures tested were pathogenic (Table 1), and the wilt symptoms produced by them were alike. These 11 cultures consisted of all the members of two of the three culturally distinct groups noted. It is believed that these two groups correspond to the two species (*Fusarium bulbigenum* var. *batatas* Wr. and *Fusarium oxysporum* f. 2 Wr.) listed by other authors as the causal organisms of the disease. As the system of nomenclature proposed by Snyder and Hansen (19) lists the

two former species as one forma, the terms group I and group II are used in this paper to distinguish between these two culturally distinct groups found in *Fusarium oxysporum* f. *batatas*.

Group I cultures are believed to be equivalent to *Fusarium bulbigenum* var. *batatas* Wr., and have the following cultural characteristics when grown on potato-dextrose agar in culture tubes (Fig. 1, A): aerial mycelium violet to white, abundant in both young and old cultures, substrate becoming dark slowly; microconidia abundant; sporodochia formed slowly,
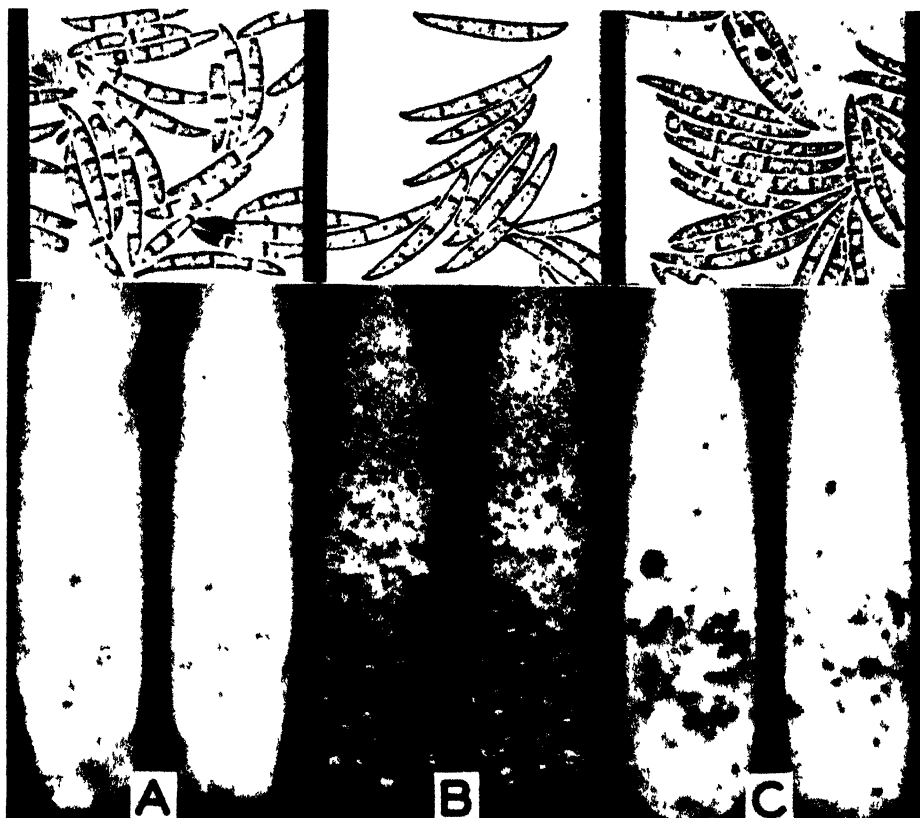
Fig. 1. Macroconidia and appearance in culture of (A) *Fusarium oxysporum* f. *batatas* group I, (B) *F. oxysporum* f. *batatas* group II, and (C) *F. oxysporum* non-pathogenic to sprouts. Spores 500×, cultures 1 month old on potato-dextrose agar.

producing macroconidia which are otherwise rare; macroconidia mostly 3- to 5-septate; 3-septate macroconidia average $35.3 \mu \times 3.4 \mu$; sclerotial-plectenchymatous masses blue, numerous, and small.

Group II cultures are believed to be equivalent to *Fusarium oxysporum* f. 2 Wr. and have the following cultural characters (Fig. 1, B) on potato-dextrose agar: aerial mycelium violet to white, abundant in young cultures, becoming sparse in old cultures; substrate becoming dark rapidly; both microconidia and macroconidia abundant, mostly 3-septate, rarely 5-

septate; 3-septate macroconidia average $33.9 \mu \times 4.2 \mu$; sporodochia formed readily, frequently occurring on sclerotial-plectenchymatous masses which are purple, numerous, and both large and small.

TABLE 1.—*Results of inoculation of freshly cut sprouts with cultures of Fusarium oxysporum isolated from sweet potato*

Culture number	Number of sprouts inoculated	Number of sprouts infected	Culture number	Number of sprouts inoculated	Number of sprouts infected
Control			Nonpathogenic cultures		
	74	0	1	5	0
Group I cultures			3	5	0
15	64	62	6	15	0
19	15	15	8	19	0
96	15	13	12	15	0
Group II cultures			13	5	0
4	15	15	20	45	0
9	20	20	27	15	0
10	15	15	39	5	0
48	20	18	46	15	0
54	54	53	47	15	0
58	15	13	50	34	0
62	20	18	60	5	0
94	15	11	83	15	0
			97	15	0

Inoculation of Healed Cuttings

In order to determine whether the pathogen could infect uninjured plants, the basal wounds were allowed to heal before inoculation. To determine the length of time necessary for healing, cuttings were set in steamed sand for various intervals and then were removed without root damage and placed in a safranin solution for 6 hr. The absence of safranin in the stem was used as an indication of complete healing, which was found to be accomplished by the formation of a callus over the wound. The time necessary for complete healing varied, presumably with the weather, being but 4 or 5 days in hot bright weather and longer with cool, overcast conditions.

In several experiments healed cuttings were inoculated with cultures representative of each group of the pathogen. Some of the plants were wounded at the time of inoculation by severing the roots or the healed base (Fig. 2), and some were left uninjured. The appearance of plants after 2 and 4 weeks is shown in figure 3. The experimental results have been consolidated in table 2.

These experiments show that the wilt *Fusarium* is incapable of penetrating healed, uninjured plants, even after considerable periods of time. Infection occurred readily where the xylem was exposed by wounding the stem or the roots, but no infection occurred via the root tips or wounds made by emergence of secondary roots.

In one experiment, not included in table 2, only one root of each healed

plant was severed a+ the time of inoculation   Of 30 plants so treated, 24 became diseased, indicating that a high percentage of infection can result from only one wound per plant.   A mild form of the disease resulted because of the unilateral infection of the stele insured by the nature of the experiment.
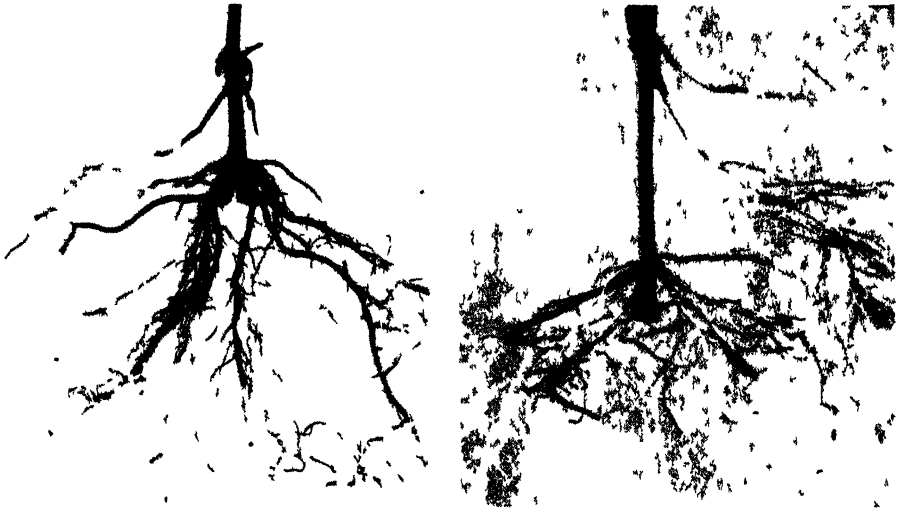
Fig 2.   Bases of sweet potato plants showing discolored roots infected with the wilt *Fusarium* via a fresh basal wound (left) and via severed roots (right).
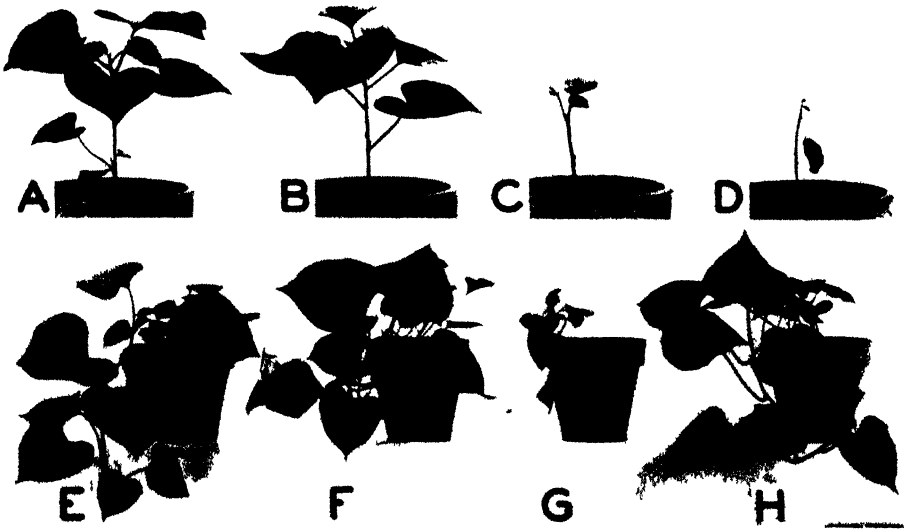
Fig 3.   Sweet potato plants grown from healed sprouts inoculated with and without wounding.   A–D.   Two weeks after inoculation, (A) noninoculated control, (B) inoculated without injury, (C) roots severed and inoculated, (D) callus severed and inoculated.   E–H.   One month after inoculation; (E), (F), and (G) same treatments as A, B, and C above using wilt Fusarium group I, (H) inoculated without injury using wilt Fusarium group II.

When fresh basal wounds were covered with an asphalt emulsion, or with sweet-potato latex, callus formation was not suppressed. However, inoculation prior to callus formation resulted in a mild infection indicating that the applied substances did not completely seal the wound.

TABLE 2.—*Results of inoculation of wounded or uninjured healed cuttings with cultures of Fusarium oxysporum f. batatas group I or group II*

Treatment of healed cuttings	Inoculum	Duration of test (in weeks)	Number of plants in test	Number of infected plants
Uninjured	None	2	15	0
		4	24	0
		8	25	0
		12	5	1[a]
	Group I	2	40	1[a]
		4	29	2[a]
		8	30	2[a]
		12	10	1[a]
	Group II	2	15	0
		4	20	2[a]
		8	15	0
		12	10	0
Base severed	None	2	4	0
	Group I	2	19	19
	Group II	2	5	5
Roots severed	None	2	4	0
	Group I	2	29	28
	Group II	2	5	4

[a] Infection traced to accidental wound on stem.

Inoculation of Vines

The sweet-potato plant is capable of sending out roots at any node that is buried in the soil even though the vine on which that node is located is still attached to the mother plant. This ability of the vines to tap down was utilized in these experiments to get roots without the necessity of having a basal wound or a basal callus. Potted healthy plants were grown until they had vines of sufficient length so that a node on the vine could be set in the soil of an adjacent pot. Before the node was covered with soil a spore suspension could be poured over the interred portion of the vine. In some cases this part of the vine was left uninjured, and in others a notch was cut into the vine, or a leaf was pulled off just before inoculation. The Jersey variety of sweet potato was found to be best adapted to this kind of experiment as it typically forms longer and more supple vines than the Porto Rico Red variety. Both varieties were used and were found to respond alike.

The results (Table 3) showed that a vascular wound was necessary for infection to take place and that root tips and wounds caused by the eruption of adventitious roots were not portals of entry for the wilt Fusarium.

TABLE 3.—*Results of inoculation of wounded or uninjured vines with Fusarium oxysporum f. batatas group I or group II*

Treatment of inoculated region of vine	Inoculum	Duration of test (in weeks)	Number of plants in test	Number of infected plants
Uninjured	None	3	23	0
	Group I	3	20	0
		4	18	0
		6	10	0
	Group II	3	5	0
		6	10	0
Stem notched	None	3	19	0
	Group I	3	25	25
Leaf removed	Group I	3	12	10
	Group II	3	12	10

Inoculation of Tobacco

As the wilt *Fusarium* from sweet potatoes has been reported able to attack tobacco (1, 18), it was of interest to determine whether the mode of infection of that suscept was the same as that found for the sweet potato. The symptoms reported to be produced by the sweet-potato wilt *Fusarium* on tobacco are those of the Fusarium wilt of tobacco which was believed by Johnson (9) to be largely dependent upon wounds for initial infection.

It was reported (18) that a Burley variety of tobacco was susceptible to two races of *Fusarium* sp., each of which could attack sweet potato, but that the flue-cured varieties were susceptible to only one of these races. Seed of a Burley variety (Kentucky Burley Improved No. 16) and of a flue-cured variety (Gold Dollar No. 15) were obtained from G. M. Armstrong.

Experiments were conducted in which both varieties of tobacco were inoculated with representatives of each of the two groups of the sweet-potato wilt pathogen. Three methods of inoculation were used; in one, seedlings were inoculated by dipping their roots in a spore suspension at the time of transplanting; in another, such seedlings were wounded by cutting their roots at the time of inoculation. The third method consisted of growing the plants from seed in pots of steamed soil, some of which were infested with spores of the pathogen at the time of planting. Neither transplanting nor cultivation was practiced, and thinning was accomplished by cutting the excess plants off at the ground level, so as not to disturb the remaining plants. At the end of an experiment each plant was surface-disinfected and examined for vascular discoloration, and isolation pieces were taken from the stem at the ground level. The results from examination and isolation corroborated each other. From table 4 showing the number of plants infected for each method of inoculation, it may be seen that the nontransplanted, uninjured plants were not infected by

either group of the pathogen, and that those plants whose roots were cut under a spore suspension showed the differential infection described by Smith and Shaw (18). The transplanted plants whose roots were not cut gave intermediate results, the amount of infection depending apparently upon the amount of root damage done by transplanting. Thus it has been found that these cultures of the sweet-potato wilt *Fusarium* are pathogenic to tobacco and that wounds exposing the xylem are necessary for infection.

TABLE 4—*Results of three methods of inoculation of two varieties of tobacco with cultures of Fusarium oxysporum f. batatas group I and group II*

Method of inoculation	Inoculum	Duration of test (in weeks)	Burley variety		Gold Dollar variety	
			Number of plants	Number infected	Number of plants	Number infected
Not transplanted (uninjured)	None	10	5	0	5	0
	Group I	10	10	0	10	0
	Group II	10	10	0	10	0
Transplanted (roots not cut)	None	6	10	0	10	0
	Group I	6	20	5	20	0
	Group II	6	20	1	20	0
Transplanted (roots cut)	None	6	15	0	15	0
	Group I	6	20	20	20	11
	Group II	6	20	11	20	0

DISCUSSION

The mode of infection of *Fusarium oxysporum* f. *batatas* is of great importance in the consideration of control practices against the disease which it causes. Experiments with cultures of this organism show that wounds involving the xylem are necessary for infection of sweet potato and tobacco. This is not in agreement with the modes of infection reported for other Fusarium wilt pathogens for which wounds are not considered to be essential. Although it has generally been considered that the wound at the base of a sweet-potato sprout is an aid to infection, it appears that infection does not normally occur via uninjured root tips, root hairs, epidermal cells, stomata, or root eruption wounds.

As the pathogen has been shown to be dependent upon vascular wounds for infection, sprout dipping should give better control than it evidently does. It may be postulated in explanation that sprouts may become inoculated prior to treatment. This does not necessarily mean invasion of the sprouts from the mother potatoes, for a common practice among growers is to freshen the sprouts by dipping the roots in water prior to treatment. The hotbed soil which is thus washed off the roots may contain spores of the pathogen and a spore suspension may result. Exposure of fresh vascular wounds to a spore suspension is the most effective method of inoculation known for this disease. Infected seed potatoes may be of importance in the infestation of hotbed soil.

The danger of accidental inoculation of sprouts may be obviated by omitting the freshening dip and by the use of sprout cuttings for direct field planting. Healing of sprouts prior to planting in the field is not feasible as a control practice because of the danger of root injury when such sprouts are transplanted.

SUMMARY

The principal mode of infection of sweet-potato sprouts by *Fusarium oxysporum* f. *batatas* was found to be by way of vascular wounds such as freshly cut stems, roots, or fresh leaf scars. Infection did not occur through uninjured stems or roots, root eruption wounds, or the callus of a healed basal wound.

Tobacco, the other known suscept of this pathogen, was infected when roots were cut or damaged by transplanting, and remained healthy when inoculated without wounding.

Single-spore isolates of the pathogen could be separated into two culturally recognizable groups which are believed to correspond to the two species listed by other authors as causing Fusarium wilt of sweet potatoes and may also correspond to two of the three races listed as causing Fusarium wilt of tobacco.

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ERWINIA ATROSEPTICA (VAN HALL) JENNISON AND ERWINIA CAROTOVORA (JONES) HOLLAND

WALTER H. BURKHOLDER AND WILSON L. SMITH, JR.

There has been a steady increase in the prevalence of blackleg of potatoes in New York State during the last few years. At present the disease, while not of importance in large areas in the potato-growing sections, is destructive in individual fields and numerous inquiries concerning it are being received from the growers. Also, further interest in the disease has arisen in connection with the problem of certification for seed potatoes. Since 1938 blackleg has been disregarded in this program (9, 16) because it was held that the disease was not seed-borne to any appreciable extent; observations, however, in New York State have not verified this supposition. When the disease appears in a field of potatoes, other fields planted to the same stock usually have varying percentages of infections; and in fields planted to potatoes from several sources, blackleg may be found in only one lot. Perhaps the most striking evidence for this last statement is contributed by the plots planted each year at Ithaca, New York, with the samples of potatoes presented for certification. Here certain samples give rise to blackleg to the amount of 6 to 7 per cent, while the remaining and surrounding samples are free from infection. The evidence all appears to be in favor of seed-borne infection, and if the disease arises from bacteria harbored in the soil it is probably unimportant in New York State.

On account of the above observations and also because a review of literature reveals so many conflicting reports on various phases of the disease, an investigation of blackleg was undertaken. The present article deals only with etiological aspects of this investigation. Such a study is important since opinions differ even on this fundamental question, and the problem has become involved in the entire subject of the soft-rot bacteria.

The first authentic description of a bacterial pathogen causing blackleg and soft rot of potatoes was given by van Hall (10) in 1902. He applied the name *Bacillus atrosepticus* to the causal organism. In the previous year, Jones (13) had described *Bacillus carotovorus*, a bacterium capable of producing soft rots in many fleshy vegetables and plant parts. From the first, the two bacteria were considered to have at least a generic relationship but it was not until years later that anyone considered them identical. Since the published description of these two pathogens, many other phytopathogenic bacteria causing rots have been reported and named and their differences and similarities recorded. Some of these bacteria have been proved to be synonyms of Jones' or of van Hall's organisms; others have been stated to be synonyms, with little or no substantiation. Various specific names, however, remain in the literature. In the sixth edition of Bergey's

Manual of Determinative Bacteriology (2), ten species of soft-rot pathogens are listed in the genus *Erwinia*, where these pathogens are now placed. No adequate proof, however, is given of the validity of most of them. Under these names also are 15 synonyms, and some dozen other soft-rot bacteria are listed, but inadequately described, in the appendix to the genus.

In recent years, Stanley (21) and Waldee (38) have thoroughly reviewed the literature pertaining to the soft-rot bacteria of the genus *Erwinia* and it will not be repeated here. It should be pointed out, nevertheless, that three schools of thought exist concerning these bacteria. Leach (15) considers that there is one species, *E. carotovora*, and that *E. atroseptica* with some six other named bacteria are synonyms of it. Thus, *E. carotovora* is held to be the cause of blackleg of potatoes. Stapp (22) concurs with Leach but applies the name *Bacillus phytophthorus* Appel to the pathogen. At the other extreme, Elrod (8), Stuart *et al.* (24, 25), Borman *et al.* (1), and, to a certain extent, Stanley (2) hold that the soft rots of plants are caused by a group of aberrant coliforms, too heterogeneous in nature for definite species separation. Waldee (28) upholds the older view of various definite species, and in a study of peritrichous phytopathogenic bacteria lists 5 species for which he establishes a new genus, *Pectobacterium*, comprising the organisms that attack the middle lamella of plant cells and produce soft rots. Thus a general confusion exists in literature as to the number of species and character of the soft-rot bacteria and as to the true cause of blackleg.

MATERIALS AND METHODS

Cultures and Isolations

Two-thirds of the bacteria used in the present investigation were isolated at the Cornell University laboratory from rotting vegetables and from blackleg lesions on potato plants. The remaining cultures were obtained from other laboratories and from the American Type Culture Collection. These cultures received from outside sources are important in that they form a connecting link between our work and the studies of previous investigators.

Beef-extract peptone agar was used as the standard medium for making isolations and for maintaining stock cultures. These species of *Erwinia* produce fairly characteristic colonies on this medium and, furthermore, remain viable for long periods of time, at least 6 months, when held at 12°C. The cultures lose their viability in a few weeks when grown on potato-dextrose agar, on other media containing sugars, and on sterilized potato plugs.

Isolation plates made from soft rots of vegetables or blackleg of potatoes, with few exceptions, yielded pathogens belonging to the genus *Erwinia* and capable of producing soft rots. The few exceptions were a brown rot of potato tubers from which a Gram-positive pathogen was always isolated; and onion rots that yielded *Pseudomonas alliicola* Burkholder and an undescribed bacterial pathogen that did not belong to the genus under study.

Associated with the pathogens in the soft-rot lesions were many saprophytic bacteria that frequently outnumbered the former. The most common types formed moderately large white colonies and were not unlike *Aerobacter aerogenes* in appearance and in their ability to reduce nitrates to nitrites, to form gas in sugar broth, and to grow well at 37° C. Attempts to produce rots in carrots and in potato tubers through artificial inoculations with these bacteria met with failure. Green fluorescent bacteria also were found not uncommonly in the isolation plates. Since vegetable rots have been reported to be caused by certain of these species, such colonies were transferred and saved whenever they appeared in any number or regularity. Of some 20-odd cultures only four had the ability to produce a soft rot of potato tubers upon inoculation; but a further study proved that the four cultures consisted of a saprophytic green fluorescent bacterium and a species of *Erwinia*.

Pathogenicity Tests

All isolates used in these experiments were first tested for their ability to infect potato tubers and cause a soft rot. The method used was that followed by Waldee (28) and others. The bacteria were placed on the surface of freshly cut tuber slices in moist chambers. Positive infection was demonstrated by the penetration of the bacteria into the tissues and the production of a soft rot. Readings were made in 24 hours, which was found to be a sufficient length of time. The method used by Elrod (8) was tried and discarded as unsatisfactory; this method is a test for protopectinase production but is not necessarily a test for pathogenicity. Certain bacteria isolated from carrots did soften carrot and potato plugs under broth but did not cause infection under normal aerial conditions. Kramer (14) demonstrated this phenomenon, and Weimer and Harter (29) have shown that saprophytic species of *Rhizopus* may produce more protopectinase than phytopathogenic ones.

Besides the pathogenicity tests on potato tubers, the isolates also were shown to have the ability to cause soft rots of carrots, cabbages, calla rhizomes, celery, cucumbers, iris rhizomes, lettuce, green pepper, onion bulbs, radishes, tender spinach, and tomato fruits. They were unable to infect rhubarb and red table beet.

Further inoculations were made on young growing potato plants in the greenhouse to determine whether the isolates could produce the blackleg disease. The variety Sebago was used mainly since it is known to be susceptible, and to develop typical symptoms of the disease. The plants were inoculated by injuring the stem at the ground level and at the same time inserting bacteria into the injury with a scalpel. Frequently the seed piece also was inoculated. The inoculated portion was then covered with moist earth, but bell glasses were not used. At least two plants were used in each test of a culture, and where infection did not take place, the experiment was repeated two and three times.

TABLE 1.—*Cultures and their sources*

Culture No.	Plant and variety	Part infected	Source	Date and by whom isolated[a]
		Erwinia atroseptica (van Hall) Jennison		
E1, E2	Potato—Warba	Stalk	New York State	1944 W. H. B.
E3, E4	Potato—Cobbler	do	do	do
E6	Potato—Sebago	do	do	do
E8	Potato—Sebago	do	Long Island	1945 W. H. B.
E14, E15	Potato—Sebago	do	New York State	1945 W. L. S.
E18	Potato	Tuber	Southern grown	1945 W. H. B.
E24	do	do	New York State	1945 W. L. S.
E25, E26	do	Stalk	Florida	1946 W. H. B.
E27	Potato—Sebago	do	New York State	do
E28	Potato—Katahdin	do	do	1946 W. L. S.
E29	Potato—Ontario	do	do	do
E46, E47	Cucumber	Fruit	do	1946 W. H. B.
E57, E58	Potato—Sebago	Stalk	Cornell greenhouse	1947 W. L. S.
E59	do	Tuber	New York State	1947 W. H. B.
E64	Carrot	Crown	do	do
E65, E66	Muskmelon	Fruit	do	do
C2	Potato		do	1945 L. C. Knorr
C12	Carrot		California	1930 P. A. Ark
C15	Potato—White Rose		do	1946? P. A. Ark
EA6	Calla		*E. aroideae* 494[b]	1929
EA7	Squash		do 7404[b]	
ES1	Potato		*B. solanisaprus*	A. Davey[c]
EP1	Delphinium		*E. phytophthora* 7403[b]	1937
EP2	Potato		do 496[b]	1906 ?
		Erwinia carotovora (Jones) Holland		
C3	Celery	Stalk	New York State	1944 W. H. B.
C6			*E. carotovora*	A. Davey[c]
C7	Iris	Rhizome	New York State	1944 W. L. S.
C8			*E. carotovora*	
C9			do 495[b]	1920?
C14	Yellow calla		California	1946 P. A. Ark
E9, E11	Lettuce		New York State	1945 W. H. B.
E19	Potato	Tuber	Southern grown	do
E20	Celery	Stalk	New York State	do
E21	Carrot	Root	do	do
E22	Potato	Tuber	do	do
E30, E31	Iris	Leaf	do	do
E32, E33	do	Rhizome	do	1946 W. H. B.
E34, E35	do	Seed pod	do	do
E37	do	Stalk	do	do
E38, E39	do	Seed pod	do	do
E40	Celery	Stalk	do	do
E43	Iris	Leaf	do	do
E55	Calla	Rhizome	do	do
E60	Potato	Tuber	Chicago market	M. A. Smith
E61	Onion	Bulb	do	do
E62	Iris	Seed pod	New York State	1947 W. H. B.
EA8	Tomato	Stem	*E. aroideae* Uganda	W. J. Dowson[c]
EO1	Cauliflower		*E. oleraceae*	F. C. Harrison[c]
ES3			*E. solanisaprus* 8061[b]	

[a] W. H. B.—W. H. Burkholder; W. L. S.—Wilson L. Smith, Jr.
[b] American Type Culture Collection number.
[c] Received through M. P. Starr.

In table 1, the 61 isolates used in the investigation are divided into two groups, based on cultural characteristics. The isolates of the first group

are considered as belonging to the species *Erwinia atroseptica* (van Hall) Jennison, and those in the second group to *Erwinia carotovora* (Jones) Holland. The first 23 isolates of *E. atroseptica* produced typical blackleg when inoculated into potato plants. Of these, E18, E46, and E47 caused excellent infection of the stem. Except that the lesions were more brown than black, the symptoms were identical with those of blackleg. The last 8 isolates of the group did not produce blackleg under our methods of inoculation. They have been in culture for varying lengths of time and could possibly have lost this ability. It should be pointed out in this connection that Waldee (28) obtained blackleg infection with two of these cultures, EP2 and EA6, some ten years ago; and that Jennison (12) also reported infection with what is undoubtedly EP2. The 30 isolates listed under *E. carotovora* in no case produced blackleg of potatoes.

Pathogenicity also was tested at various intervals during the investigation to determine whether the isolates were still capable of producing the blackleg disease and the rot of vegetables. The ability to rot vegetables probably is seldom lost, if we may judge by the performance of C9, EP2, and EO1 which have been in culture approximately 28, 42, and 46 years respectively.

Pure Culture Studies

Before conducting the cultural and biochemical studies, all isolates were purified two and three times by replating. Furthermore, whenever there was any doubt concerning the purity of a culture during the progress of the work, it was again replated. Little or no variation appeared among the 31 isolates of *Erwinia atroseptica*. Greater variation was shown among the *E. carotovora* isolates than those of *E. atroseptica*, but even here the differences, which were never great and were usually in degrees of reaction, could not be considered varietal differences. A culture of *Aerobacter aerogenes* was carried with the soft-rot bacteria for comparison. The difference between this species and the *Erwinia* species was always distinct, hence there is no danger of confusing them.

The methods employed in preparing the culture media and making the biochemical tests followed in these studies may be found in the latest editions of the Manual of Methods for Pure Cultural Study of Bacteria (3) and in the Difco Manual of Dehydrated Culture Media and Reagents (4). Where a deviation occurs from these methods it is so stated. All cultures unless otherwise mentioned were incubated at 27° C.

In conducting the fermentation experiments we used the basal medium of Koser's citrate medium, that is, the inorganic salts, to which were added aseptically the various carbon compounds. Sugars, alcohols, and glucosides were added at the rate of 1 per cent and the organic acid salts at 0.15 per cent. The sugars were sterilized by passage through a Seitz filter. Brom thymol blue was used as an indicator except where the use of brom cresol purple is indicated.

ERWINIA ATROSEPTICA (VAN HALL) JENNISON

The following description of the pathogen is based on a study of the 31 isolates listed in table 1. The specific name used follows the work of Paine (19), Morse (18), and Jennison (12).

Morphology and Staining Reactions

The pathogen is a non-spore-forming rod with rounded ends, approximately 1.5μ (1–2μ) by 0.7μ. It is Gram-negative, and motile with peritrichous flagella (Casares-Gil's stain). The number of flagella varies from 1 to 6. Cells may frequently have a central granule. At times old cultures such as EP2 may lose their motility, and flagella cannot be demonstrated with the stain.

Cultural Characters

Beef-extract peptone agar (Bacto). Growth in 24 hr. is moderate, filiform, grayish white and iridescent, slightly moist, and butyrous. The medium remains unchanged in color.

Broth. Moderately turbid with slight ring, but seldom a pellicle. Later a white sediment is formed.

Litmus milk. Coagulation after 4 days. Some isolates are slower. Litmus is reduced and there is a slight separation of whey, but little or no peptonization. Cultures held at room temperature in this medium for 7 months were not viable.

Potato plugs. Growth is slight in 48 hr. with little subsequent increase. Tip of plug sometimes becomes dark brown.

Endo agar plates. (Bacto). Colonies circular, 2 mm. in diameter; pink at first, becoming red and later covered with gold metallic luster.

Krumwiede triple sugar agar (Bacto). Growth good. Medium turns yellow in 48 hr. Viability poor.

Uschinsky's solution. Light turbidity in 2 days, with rapid settling of cells.

Methylene blue milk. A 0.01 per cent methylene blue reduced in 2 days.

Desoxycholate agar. Good growth. Colonies turn pink.

Biochemical Reactions

Growth in gelatin stabs appears in 24 hr. at 21° C. Liquefaction begins the second day and proceeds at a moderate rate. Indole is not formed in tryptone broth, nor is H_2S detectable in this medium with lead acetate paper. Neither is there an indication of H_2S production in triple sugar iron agar. In lead acetate agar stabs, however, a slight darkening appears. Nitrates are reduced to nitrites in the synthetic medium in 2 days. Sodium selenite also is reduced (0.1 per cent in a glucose-yeast extract agar). The methyl red test is positive, the Voges-Proskauer tests negative, with the exception that E65 and E66 gave a slight reaction. A limited number of tests for lipolysis were negative when the Starr method (23) was used. Five

per cent sodium chloride when added to beef-peptone broth causes a decided reduction in growth; seven per cent inhibits growth.

Carbon utilization studies. The writers are in agreement with Harding and Morse (11), Waldee (28), and others that the *Erwinia* soft-rot pathogens are microaerogenic. In the present studies certain isolates always produced a small amount of gas in various sugar broths. Other isolates produced gas with no regularity and several cultures of both pathogens, E46, E47, E60–E62 and E64–E66 have remained negative for this character. Lactose formate ricinoleate broth was used with variable results. Beef-peptone or synthetic broth when used as a basal medium played no part in gas production, nor did the temperatures 21° C., 27° C., and 31° C. Furthermore, no correlation could be observed between gas production and other cultural characters of the two organisms under discussion.

The utilization of the carbon compounds, however, is a dependable character, and may be used in a description of the organism. *Erwinia atroseptica* utilizes the following with an acid production except with the salts of the organic acid, where an alkali is usually produced: glucose, galactose, levulose, arabinose, xylose, rhamnose, cellobiose, lactose, maltose, raffinose, sucrose, glycerol, mannitol, salicin, and sodium ammonium pectate[1]; also the sodium salts of citric and lactic acid. It does not utilize ethyl alcohol, ducitol, erythritol, nor the sodium salts of hippuric, malonic, tartaric, or uric acid. Starch is not hydrolyzed, nor is cellulose attacked.

Since the fermentation of lactose is an important character in the family Enterobacteriaceae, the following observations are noted. Acid is formed in 48 to 72 hr. with most of the isolates. Gas when produced appears later. Exceptions were EA6 and EA7, which were about 2 days slower in acid production, but they produced gas in this sugar, especially in lactose formate ricinoleate broth.

Nitrogen relations. *Erwinia atroseptica* utilizes ammonium salts, potassium nitrate, peptone, gelatin, and yeast extract, but not aspartic acid. It can utilize asparagine as a combined carbon and nitrogen source. Tyrosine is not so used. Whether or not the pathogen utilizes this amino acid as a nitrogen source is not known, but a melanin reaction was never observed when tyrosine was added to a complete nutrient medium. The pathogen is urease negative by the method of Stuart *et al.* (26).

Optimum temperature is approximately 27° C.; minimum, 3° C.; while the maximum varied somewhat with the isolate. All grew at 31° C., a few did not grow at 32° C. and a few gave a light growth at 35° C., but none grew at a higher temperature. The freshly isolated strains are the most sensitive to high temperatures.

ERWINIA CAROTOVORA (JONES) HOLLAND

The following description is based on the study of 30 isolates listed in table 1. This soft-rot bacterium is very similar to the blackleg pathogen

[1] Product 24 of California Fruit Growers' Exchange, Ontario, California.

and only its cultural characters which are different from those of *Erwinia atroseptica,* as presented above, are set forth here. Attention is called, however, to the minor variations that are found within *E. carotovora.*

Morphology and Staining Reactions

The flagella of *E. carotovora* take the stain more readily than do those of the blackleg pathogen.

Cultural Characteristics

Broth. Certain of the isolates, but by no means all, produced a greater turbidity than did *Erwinia atroseptica.*

Starch agar. *E. carotovora* does not have as great a tendency to form spreading colonies on this agar as does the blackleg pathogen.

Krumwiede triple sugar agar. The agar becomes yellow due to an acid reaction, but later certain of the isolates reverse the process and the agar takes on a red color.

Biochemical Reactions

Methyl red and Voges-Proskauer tests. Most of the isolates were methyl red positive, a few were doubtful, and several were negative. The categories varied somewhat in separate tests, so definite figures are impossible. Again, in the Voges-Proskauer test the majority were unable to produce acetyl-methyl-carbinol. A few did produce this compound and others varied in this respect. Waldee (28) obtained similar results.

Carbon utilization studies. *Erwinia carotovora* utilizes, in addition to those compounds listed as utilized by *E. atroseptica,* the following: ethyl alcohol, dulcitol, sodium hippurate, sodium malonate, and sodium urate. Growth in the last was rather meager. In one test *E. carotovora* utilized erythritol, while the blackleg pathogen did not. In the utilization of these compounds we have a very definite distinction between the two species.

The use of 5 per cent ethyl alcohol in beef-extract peptone broth with brom thymol blue as an indicator is a reliable medium to separate *Erwinia atroseptica* and *E. carotovora.* Since certain commercial alcohols contain viable bacterial spores which must be eliminated, the alcohol should be filtered and added aseptically after the broth is sterilized. *E. carotovora* turns the indicator medium first alkaline (blue), then in approximately a week, acid (yellow). With *E. atroseptica* the medium turns alkaline and remains so.

The use of maltose and of glycerol has been advocated by several investigators as a means of separating these two species of *Erwinia.* Dowson (5, 6) used maltose, Waldee (28) glycerol, for this purpose. Elrod (7) also found maltose an aid in separating the groups of soft-rot pathogens in this genus. In the present investigation it was determined that both species were able to utilize the two compounds with varying amounts of acid production. *E. carotovora* when grown in a synthetic medium plus maltose produces moderate turbidity, but acid production is so slight it is not registered

by the brom cresol purple in the medium.   On the other hand, many of the isolates of *E. atroseptica* with little, or any, greater turbidity produce sufficient acid to turn brom thymol blue or even brom cresol purple to yellow.   With the use of glycerol all isolates produce a sufficient amount of acid to turn brom thymol blue to yellow in a synthetic medium.   When peptone is added as in the Waldee medium (28), *E. atroseptica* produces good growth, but little acid, and no color change with brom cresol purple. Certain isolates of *E. carotovora*, however, produce sufficient acid from glycerol so that this indicator turns yellow.   Thus a difference may be noted at times between the two species when grown in a maltose or glycerol broth. The difference is one of degree, however, and certain isolates vary in their ability to produce the acid.   The tests are, therefore, not too reliable.

Temperature relations.   *Erwinia carotovora* has a somewhat higher temperature range than does *E. atroseptica.*   Few of the isolates would grow below 6° C.   All grew at 35° C., and two made a slight growth at 37° C. There is some overlapping of the two species at 35° C.

<center>DISCUSSION</center>

In the present investigation a sufficient number of isolates of the soft-rot bacteria of the genus *Erwinia* have been studied to reach certain definite conclusions.   Also, a fairly extensive survey has been made of the characteristics of these bacteria that go beyond generic characters.   The 61 isolates used were of proved pathogenicity and of different ages and from different localities, and came from various plants and plant parts, but they readily fell into the two species, *E. atroseptica* and *E. carotovora.*   The former appears to be a fairly stable species, since all such cultures were practically identical regardless of their history.   *E. carotovora* isolates showed certain slight variations but not such as could be considered varietal differences.

During the investigation none of the isolates showed any indication of losing in culture the ability to produce a soft rot.   Very old cultures also rotted potato tubers and carrots as readily as did recent isolates.   *Erwinia atroseptica*, however, may lose its ability to infect growing potato plants and cause the blackleg symptoms.

The separating characters we have used for the two species are not all new.   Dr. E. F. Smith (20) and Massey (17) both recognized that ethyl alcohol utilization was a distinguishing character.   We have discussed maltose and glycerol in relation to their use by other workers, and we believe they are not to be used with too much reliance.

The fact that we were unable to find a culture of a soft-rot bacterium among our own isolates or those received from other laboratories that we considered *Erwinia aroideae* is interesting.   Most investigators agree that species of *Erwinia* causing soft rot are microaerogenic, and at times gas is not produced by either of the two species dealt with here.   The character, therefore, is not a reliable one in distinguishing *E. aroideae.*   Townsend

(27) gives only one other separatory character that can be used with certainty; that is, tolerance to temperatures higher than those in which *E. atroseptica* or *E. carotovora* can grow. He states that the maximum temperature is 41° C., which is considerably above the maximum for the other two species. Townsend also reports production of H_2S in lead-acetate agar, but here the character might be subject to interpretation. From our work we cannot state with certainty that *E. aroideae* is or is not a valid species. Three cultures that were received and labeled as such produced gas; two had the characteristics of *E. atroseptica* and one of *E. carotovora*. Waldee (28) uses the name, however, and places A.T.C.C. 494 in this species although he reports that it causes blackleg of potatoes. We have placed this culture in *E. atroseptica* since it has the characters of the blackleg organism.

The 61 soft-rot pathogens included in the two species and dealt with in this article cannot be considered aberrant coliform bacteria. The "plant coliform cultures" of Stuart *et al.* (24, 25), Borman *et al.* (1), and Elrod (8) may be considered as such. No adequate proof has been given of their pathogenicity; furthermore, their ability to grow well at 37° C. throws doubt on the possibility of these being species of *Erwinia*. They appear similar to the saprophytes referred to earlier in this article.

SUMMARY

Sixty-one isolates of *Erwinia* were studied in this investigation. They could be divided into two species, *E. atroseptica* and *E. carotovora*. The former appears to be a more stable species than the latter.

E. atroseptica can produce the symptoms of blackleg in potato plants. *E. carotovora* can not.

The two species may be distinguished in culture on the utilization of carbon compounds. *E. carotovora* utilizes ethyl alcohol, dulcitol, sodium hippurate, sodium malonate, and sodium urate. *E. atroseptica* does not.

Other characters that aid in separating the two species are their action on maltose and glycerol, their temperature relations, and their growth on starch agar.

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DEVELOPMENT OF ANTHRACNOSE AND SECONDARY ROTS IN STORED TOMATO FRUITS IN RELATION TO FIELD SPRAYING WITH FUNGICIDES

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Anthracnose or ripe-rot of tomato, caused by *Colletotrichum phomoides* (Sacc.) Chester, is one of the most destructive diseases of this crop. In addition to the losses from fruit rotting in the field, a high mold count in processed tomatoes may result from blending a very small percentage of anthracnose-affected fruits with those free from the disease (8). Thus, even a trace of anthracnose may prove costly to both grower and processor because of the additional time required for picking, sorting, and trimming. Although anthracnose is not of great importance in regions where tomatoes are harvested green and packed for shipment (2), and is found only occasionally on fruits in transit and in ripening rooms (10), the disease may be at times an important factor in the retail marketing of such fruits. A high percentage of infection was noted by the senior author on southern-grown "green-wraps" displayed in stores in Urbana, Illinois, in April 1948 and again in St. Louis, Missouri, in January 1949. Over 50 per cent of the fruit in one lot was rendered unsalable from anthracnose alone.

Anthracnose lesions on mature[2] fruits often arise from infections which occur while the fruits are still green. Following penetration the fungus may remain dormant under the cuticle for as long as 3 or 4 weeks (3). As the fruits approach maturity, growth of the pathogen is resumed and surface lesions appear (3, 11). Development of anthracnose is especially rapid in cannery-red tomatoes after picking. Such fruits often develop numerous lesions if allowed to stand overnight in hampers.

Defoliation of tomato plants appears to favor the development of anthracnose (5). The close association between incidence of defoliation and anthracnose has been attributed (8) to similar weather conditions favoring both leaf-spot fungi and anthracnose or to increased anthracnose susceptibility of fruits on defoliated plants. Wilson and Runnels (15) found that fruit on early-maturing and early-defoliating, determinate (self-topping) tomato varieties are more subject to anthracnose than those on later-maturing and later-defoliating, indeterminate (not self-topping) kinds. They concluded that early contact of the fruit with the ground and excessive exposure to sunlight increase susceptibility to anthracnose.

The ideal fungicide for the control of anthracnose should have a high degree of specificity not only for the pathogen causing this disease but also for the leaf-spot fungi. However, very few fungicides give good control of both defoliation and anthracnose. Fungicides containing copper are in

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[2] Terminology for fruit maturation and ripening recommended in (7).

general reasonably effective against defoliation diseases but give rather poor control of anthracnose (8, 12). The zinc salts of carbamic-acid derivatives such as "Zerlate," "Dithane D-14" plus zinc sulfate, "Dithane Z-78," and "Parzate" appear to be efficacious against early blight and anthracnose but not against Septoria leaf spot (1). The ferric salts such as "Fermate" give good control of anthracnose but appear inadequate against defoliation diseases (8, 12).

Practically all of the data reported thus far on anthracnose control with fungicides have been taken at harvest and do not include disease development in storage. Yet heavy losses may occur in the interval between harvest and processing, especially at the peak of the season when it may be necessary for tomatoes to remain in hampers for several hours or even days. McNew (8) reported an increase of about 20 per cent in anthracnose on unsprayed cull fruits left on the ground for a week after harvest compared with only 6 per cent on Fermate-sprayed fruits. He pointed out that many of these discarded fruits became infected while on the ground. However, the increase in infection during this period was "closely correlated with the amount of infection in each spray treatment at the beginning of the period." Wilson (16) stored fruits for 2 and 4 days after harvest in measuring varietal resistance among tomatoes to anthracnose.

This paper presents results of investigations at Urbana, Illinois, from 1945 through 1948 on the effect of spraying tomatoes with various fungicides, used alone and in alternating schedules with others, on the incidence of anthracnose in fruits at harvest and after storage. Data were obtained also on the development in storage of secondary rots caused primarily by soil-borne organisms and on the control of early blight caused by *Alternaria solani* (Ell. and G. Martin) Sor. The general fungicide tests were run in conjunction with cooperative experiments among several States.

MATERIALS AND METHODS

Several organic and inorganic, copper and zinc fungicides were employed in tests during the 4-year period. These fungicides and concentrations were: "Bioquin 1" (copper 8-quinolinolate)—1 lb./100 gal. water; Bordeaux mixture—8/8/100; "Dithane D-14" (disodium ethylene bisdithiocarbamate) and zinc sulfate—2 qt./1 lb. 100; "Dithane Z-78" and "Parzate" (zinc ethylene bisdithiocarbamate)—2/100; "Fermate" (ferric dimethyl dithiocarbamate)—2/100; Tribasic copper sulfate—4/100; Yellow Cuprocide (yellow cuprous oxide)—1.5/100; and "Zerlate" (zinc dimethyl dithiocarbamate)—2/100. "Triton B-1956," a wetting agent, was added to each spray mixture at the rate of 3 oz. to 100 gal. Sprays were applied with a single-nozzle, orchard-type gun (6/64-in. disk-aperture) adjusted to give a 40–50° cone at 200 lb. pressure and at approximately 200 gal. to the acre. Applications were made on the average at 10-day intervals beginning about 4 weeks after the appearance of the first crown-cluster flowers. In schedules involving Tribasic copper sulfate alternating with "Zerlate" or with "Fermate," the dithiocarbamate was

used for the first, third, and fifth applications and Tribasic for the second and fourth. The fifth application of all fungicides was omitted in the 1946 experiments.

An early, determinate variety (Illinois T-19)[3] was used in 1945 and a later, indeterminate kind (Garden State) in subsequent years. Ten-plant plots replicated five times were laid out for each treatment. Fruits were picked at approximately weekly intervals and graded into U. S. No. 1, No. 2, and culls. The number and weight of anthracnose-affected fruits which were laid to one side during grading were recorded. These fruits were then recombined with unaffected fruits and the total number and weight in the three grades recorded. Prior to 1948, fruits with only one lesion were classified as No. 2 if they otherwise fell into this category. Those with two or more lesions were considered as culls. In the 1948 tests all infected fruits were classified as culls regardless of number of lesions.

The No. 1 fruits from each treatment[4] were graded further for storage tests into an "extra fancy" selection consisting of those almost completely free from growth cracks and from mechanical injuries received during picking and grading. These precautions were taken to prevent, insofar as possible, the development in storage of secondary rots which might result in premature termination of the tests. The reselected samples from each treatment consisted of a minimum of 60 fruits. With the exception of one test in 1945 involving tomatoes harvested at the turning (pink-blush) stage, all fruits selected for storage were mature (cannery-red) so far as could be determined from skin coloration.

Tomatoes in the 1945 and 1948 tests were stored in clean hampers indoors at temperatures which fluctuated between 65° and 80° F., while those in the 1946-47 experiments were left in uncovered clean hampers in the field. During this 7-day storage in 1946 rainfall totaled 0.42 in. on two consecutive days and air temperatures averaged 63° F. (high, 87°; low, 38°). In 1947, rainfall was recorded as a "trace" on one day out of the 5-day storage period with an average air temperature of 76° F. (high, 85°; low, 67°). Each fruit in all tests with the exception of those in 1947 and in one test in 1948 (August 26) was examined one or more times before termination of storage. Tomatoes affected with anthracnose or secondary rots at this time were counted and removed from the hampers to retard fruit-to-fruit spread of the causal organisms. No surface moisture was evident at any time on fruits stored indoors.

<div align="center">RESULTS</div>

The relative efficiency of the several treatments in controlling anthracnose as determined from harvest records was not changed materially by holding No. 1 fruits in storage (Table 1). This corroborates McNew's

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[3] This variety, under the name "Huelsen," was claimed by Wilson (13) to be very susceptible to defoliation from early blight and to anthracnose.

[4] Fruits were taken from only one or two replicates in 1946 and 1947, and from each of the five replicates and combined in one basket in 1945 and 1948.

TABLE 1.—*Incidence of tomato anthracnose and secondary fruit rots* at harvest and after storage in relation to field spraying with fungicides and to defoliation from early blight. Illinois T-19 variety used in 1945 and Garden State in 1946-48*

Year[b] and treatment[c]	Percentage anthracnose			Percentage secondary rots after storage	Percentage defoliation[e]
	At harvest	After storage	Total[d]		
1945 (1)					
Fermate-Tribasic	1.9	4.0	5.8	10	0
Zerlate	0.5	6.0	6.5	10	0
Fermate	5.5	15.0	19.7	15	11
None	15.4	42.0	50.9	7	76
L.S.D.[f]	7.3				5
1945 (2)					
Fermate-Tribasic		23.1		44	5
Zerlate		25.9		43	17
Fermate		37.5		43	57
None		54.9		41	100
1946					
Zerlate-Tribasic	5.0	20 5	24.5	36	22
Zerlate	1.1	23.2	24.0	37	27
Fermate-Tribasic	7.9	28.6	34.2	31	27
None	35.0	67.6	78.9	13	85
1947					
Zerlate	0.0	2.0	2.0	38	2
Zerlate Tribasic	1.0	4.0	5.0	42	2
Cuprocide	8.6	18.0	25.0	32	6
Z-78	8.0	22.0	28.2	32	6
Tribasic	13.5	22.0	32.5	32	2
None	9.9	34.0	40.5	24	45
1948 (1)					
D 14	4.4	4.0	8.2	60	Trace
Parzate	6.3	12.0	17.5	42	do
Z-78	17.7	16.0	30.9	47	do
Zerlate	20.8	34.0	47.7	28	do
Zerlate-Tribasic	28.4	37.0	54.9	24	do
Tribasic	48.7	48.0	73.3	13	do
Bordeaux	43.1	51.0	72.1	18	do
Bioquin 1	40.6	52.0	71.5	22	do
None	72.1	g			20
L.S.D.	8.6				
1948 (2)					
Bioquin 1	8.7	19 0	26.0	32	2
Zerlate-Tribasic	6.7	22.0	27.2	29	6
D-14	10.1	26.0	33.5	43	1
Parzate	23.6	37.0	51.9	46	3
Z-78	20.2	40.0	52.1	21	2
Zerlate	12.5	42.0	49.2	19	7
Bordeaux	40.3				7
Tribasic	54.7				12
None	77.5				67
L.S.D.	6.6				3

* Rots due primarily to soft-rot bacteria, *Rhizopus* sp. and *Oospora* sp., in descending order of importance.

[b] Dates of harvesting, and storage of fruits: 1945 (1)—Sept. 21, mature, 6 days indoors; 1945 (2)—Oct. 5, immature, 10 days indoors; 1946—Sept. 27, mature, 7 days outdoors; 1947—Sept. 4, mature, 5 days outdoors; 1948 (1)—Aug. 26, mature, 3 days indoors; 1948 (2)—Sept. 15, mature, 5 days indoors.

[c] Spraying dates: 1945—July 27, Aug. 9, 17, 27, Sept. 7; 1946—July 23, Aug. 5, 19, Sept. 3; 1947—July 24, Aug. 4, 15, 27, Sept. 5; 1948—July 9, 19, 30, Aug. 6, 18.

[d] Minimum total percentage $= \dfrac{(100 - AH) \times AS}{100} + AH$ where AH = number of infected fruits at harvest and AS = number developing symptoms in storage.

[e] Defoliation records taken 5 days before or after harvest.

[f] Least difference necessary for significance at 5 per cent level but given only if fruits were taken from all replicates.

(8) findings with cull fruits left in the field after harvest. However, anthracnose infections developed more abundantly in storage in certain lots than in others. For example, in the first 1948 experiment there was no increase in anthracnose in stored fruits sprayed in the field with D-14, Z-78, or Tribasic copper sulfate, in contrast to an appreciable increase in fruits sprayed with other fungicides. In general there was but little difference between Zerlate alone and Tribasic alternating with Zerlate or Fermate. The degree of control on stored fruits was particularly high where the period between date of last spray and harvest was 2 weeks or less (September 1945–46, August 1948), and somewhat poorer where this period was longer than 3 weeks (remainder of tests). Unfortunately no comparisons can be made with unsprayed fruits in the 1948 tests since not enough anthracnose-free tomatoes were available from these plots for storage. Dithane Z-78 was comparatively poor in the 1947 experiments but its rating was higher the following year. Dithane D-14 was superior in anthracnose control to all other fungicides in the first 1948 test and was still near the top in the second test. Parzate was somewhat better than Z-78 and Zerlate in the August 1948 test. There was but little difference, however, among these fungicides approximately 3 weeks later. The 1947–48 experiments demonstrate again the inadequacy of most of the copper fungicides in controlling anthracnose. Bioquin 1 presents somewhat of a paradox in that it was the poorest fungicide of all in the August 1948 test but was at the top in the later experiment. The same thing is true to a lesser degree for Zerlate alternating with Tribasic. This reversal of position is being investigated further.

The inoculum potential was fully expressed only in the September 1945 test where no new lesions developed on Zerlate and Fermate-Tribasic-sprayed fruits between the fifth and sixth days of storage. In all other tests, measurement of the anthracnose potential was not possible because of the development of secondary rots and physiological breakdown which resulted in termination of storage.

Defoliation apparently had some effect on the incidence of anthracnose at harvest in all tests but it was least apparent in those of 1947 and 1948. Rainfall for August 1947 was 1.48 inches below normal and temperatures 7.4 degrees above normal. There was but little defoliation even in the control plots and likewise only a small amount of anthracnose. In fact, infection in these plots was somewhat less than in those sprayed with Tribasic copper sulfate. There were a large number of incipient infections, however, and these became apparent when the fruits were placed in storage after harvest. Rainfall for August 1948 was 1.54 inches below normal but temperatures were only 0.6 degrees above normal. Defoliation was negligible in all but the unsprayed plots in the August test. The severe anthracnose infection appeared to develop independently ·of defoliation. Storing fruits from the various plots for 3 days did not result in any large increase in anthracnose indicating that there were only a few incipient

lesions. Defoliation had increased only slightly in the sprayed plots by September 15. The number of lesions increased appreciably when the fruits were stored, suggesting that new infections had occurred in the 3-week interval between tests.

Secondary rots were consistently worse on stored fruits from sprayed plots than on those from unsprayed plots in the 1945–47 tests. However, these rots did not appear to be much worse in one treatment than in another. In the August 1948 experiment, fruits sprayed with the zinc ethylene bisdithiocarbamates (D-14 plus zinc sulfate, Z-78, and Parzate) had the greatest, and those with copper fungicides the least amount of rotting. The situation in the September test was somewhat similar but not so striking because no stored fruits sprayed with Bordeaux mixture and Tribasic were available for comparison. In this instance fruits sprayed with Z-78 developed less secondary rot than those sprayed with D-14 and Parzate. In both tests Zerlate appeared to be the best of the organic zinc fungicides for controlling secondary rots.

An attempt was made in one test (September 1945) to determine the principal infection court of these secondary rot organisms. A total of 85 per cent of secondary rotting in stored fruits apparently originated from mechanical injuries resulting from handling and the remainder from minor growth cracks.

DISCUSSION

The incidence of anthracnose at harvest may be more a function of fungicidal efficiency against both defoliation diseases and anthracnose than against anthracnose alone. Severely defoliated plants yield a relatively high percentage of soft, off-color, cull tomatoes because these fruits have been exposed to direct sunlight and high temperatures. Such fruits are often more severely affected with anthracnose than tomatoes on undefoliated plants. Therefore, it would appear virtually impossible to evaluate accurately fungicides for anthracnose control from harvest data alone, unless anthracnose develops in the absence of defoliation as in the 1948 tests. Such a situation is extremely rare.

The near-maximum anthracnose potential can be determined and the complicating effect of defoliation largely eliminated by holding No. 1 (cannery-red) fruits in storage after harvest. All such fruits, regardless of the exact stage of maturation when harvested, eventually reach post-ripeness which is more favorable for symptom expression than immaturity at harvest. The speed with which incipient lesions can develop in storage would depend largely upon temperature, the optimum for growth of the pathogen in mature fruits being around 80° F. (3, 9).

The stage of maturation of red-fruited tomato varieties, to say nothing of yellow-fruited kinds, can scarcely be determined from skin coloration alone. Therefore, storing fruits after harvest might provide a reliable way for evaluating varietal resistance to anthracnose. Fulton found[5] that an-

[5] Unpublished data from University of Illinois thesis.

thracnose-inoculated, greenhouse-grown, yellow-fruited Jubilee tomatoes, picked and stored when apparently mature, did not develop lesions as rapidly as did red-fruited Early Baltimore and Garden State. In time all three varieties showed approximately the same number of lesions per fruit.

The reason for secondary rots being worse on sprayed than on unsprayed fruits is not apparent from these data. Sprayed fruits might be more easily injured in handling than unsprayed. It is interesting to note in this connection that Wilson and Runnels (15) ranked Garden State and Illinois T-19 as 38th and 45th respectively among 48 varieties tested for resistance of the fruit epidermis to mechanical puncture. It may be that exposure to direct sunlight directly or indirectly results in toughening of the skin of fruits on defoliated plants or that initial infection by rot organisms is favored by the microclimate under comparatively undefoliated plants. Horsfall and Heuberger (4) were unable to demonstrate any relationship between defoliation and infections originating from soil-borne organisms. The fruits in their experiments were examined presumably at harvest and not after storage.

The wide variation in secondary rotting in the 1948 tests where defoliation was not an important factor may have been due to differences among fungicides in fungitoxicity or in phytotoxicity. An analysis of the incidence of culls[6] (excluding anthracnose-infected fruits) at harvest in both tests showed that no fungicide was significantly better than another in reducing the number of cull fruits. In stored fruit, however, consistently fewer cull fruits were obtained with Bioquin 1[7] and Tribasic copper sulfate than with any other fungicides including Bordeaux mixture. The copper fungicides as a whole permitted less secondary rotting than did the organic zinc fungicides. From the standpoint of phytotoxicity, Dithane D-14 and Parzate were the only fungicides causing any visible foliage injury, namely, a yellowing limited to the extreme margins of the leaflets. Injury to tomato foliage by these materials has been noted in other experiments (1). However, there is no proof that injury in this instance contributed in any way to fruit rotting.

From these tests it is apparent that tomato growers and processors can obtain appreciable control of anthracnose even up to the time the tomatoes are canned or processed if the proper fungicides are used. Although no one fungicide will give adequate control at present of all tomato diseases, it would appear that Zerlate alone and Zerlate alternating with Tribasic copper sulfate are somewhat superior to the other fungicides tested in controlling all of the diseases prevalent in these experiments.

SUMMARY

Studies were made over a 4-year period on the development of anthrac-

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[6] Culls were made up almost entirely of fruits affected with rots due to various species of fungi and bacteria with only a trace of *Rhizopus* sp., *Rhizoctonia* sp., and *Phytophthora* sp.

[7] Wilson (13) obtained with Bioquin 1 unusually good control of fruit rots other than anthracnose on the basis of harvest data.

nose and secondary rots in tomato fruits sprayed during the season with several different fungicides. Data were taken on extent of defoliation from early blight, on the incidence of anthracnose at harvest, and on the amount of anthracnose and secondary rots in selected U. S. No. 1 fruit held in storage for several days after harvest.

The efficiency ratings of the various fungicides in controlling anthracnose based on harvest data were not changed materially as a result of holding fruit in storage. However, in a few instances anthracnose development in storage was more pronounced in certain lots than in others. In general, Zerlate alone, Tribasic copper sulfate alternating with Zerlate or Fermate, and Dithane D-14 plus zinc sulfate were somewhat superior to other fungicides in controlling anthracnose. In one experiment Bioquin 1 and Tribasic copper sulfate alternating with Zerlate gave relatively poor control in the latter part of August but were better than all other fungicides 3 weeks later.

With the exception of one experiment, measurement of the maximum anthracnose potential was not possible because of secondary rots and physiological breakdown resulting in termination of storage.

Defoliation was apparently an important factor in the development of anthracnose in most of the experiments. However, severe anthracnose infections developed in 1948 in the almost complete absence of defoliation.

Secondary rots in stored tomatoes due to soil-borne bacteria and fungi were worse in sprayed than in unsprayed fruit. Most of these pathogens appeared to gain entrance principally through mechanical injuries. In one test where defoliation was not a factor, the dithiocarbamates were considerably less effective in controlling these rots than fungicides containing copper. It is not apparent whether this resulted from differences in fungicidal efficiency or from variance in phytotoxicity.

Fungicidal efficiency against anthracnose can be measured more accurately by determining incidence of this disease on No. 1 fruit after storage than by depending on harvest records alone. Storage of such fruit reduces to a minimum the related factors of defoliation and exposure of fruit to high temperatures which make the fruit more susceptible to anthracnose. Storage also would appear to be useful in evaluating varieties for anthracnose resistance in view of the fact that the stage of fruit maturation is difficult to determine from skin coloration alone.

These experiments demonstrate that tomato anthracnose can be controlled up to the time the fruit is processed if the proper fungicides are used.

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A STUDY OF LINKAGE BETWEEN FACTORS FOR RESISTANCE TO WILT AND NEAR-WILT IN GARDEN PEAS

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INTRODUCTION

The existence of wilt (*Fusarium oxysporum* f. *pisi* (Linford) race 1 Snyder and Hansen) (7) of pea (*Pisum sativum* L.) was first recorded in Wisconsin by Jones and Linford (3) in 1925. A number of standard varieties were found to be mixtures of resistant and susceptible plants. Reaction to wilt was reported by Wade (9) in 1929 to be governed by a single factor pair with resistance dominant. After wilt-resistant varieties of peas were generally adopted, another wilt disease appeared (6), similar to the first in symptoms but slower in development. This disease was named near-wilt (*Fusarium oxysporum* f. *pisi* (Linford) race 2 Snyder and Hansen) (7) and was fully described by Snyder and Walker (8) in 1935. Resistance to near-wilt was found and was reported by Hare *et al.* (1) in 1949 to be caused by a single dominant factor.

Genetic interest in these two fusarial diseases of peas first centered upon the mode of inheritance of reaction and then upon the possibility of linkage between the factors governing reaction to them. The object of this paper is to report a study of association between the factors determining reaction to the wilt and near-wilt diseases of peas.

METHODS AND MATERIALS

The data for this study were obtained from crosses of Delwiche Commando with Davis Perfection and with Thomas Laxton. Delwiche Commando is resistant and the other two varieties are susceptible to wilt and near-wilt. The crosses were made in the summer of 1946. Soon after harvest, part of the hybrid seed was planted in the field and part in the greenhouse. Seeds were obtained subsequently from both selfing and backcrossing the F_1 plants. The first generation from selfing and the first generation from backcrossing were grown in the field in 1947 and seeds obtained from these plants were seeded for testing for plant reaction to wilt and near-wilt in the greenhouse during the winter season of 1947–48.

The seeds from each plant were divided into two equal portions, one for each of the two disease tests. A maximum of 24 seeds per line was seeded for each test, but many lines had fewer than that number. Tests were made for wilt reaction in sterile white quartz sand in 6-in. clay pots set on open benches in an air temperature high enough to maintain a sand temperature of about 22° C. The sand was flooded with distilled water in preparation

for planting. Inoculum of the organisms inciting both wilt and near-wilt was obtained in the manner described elsewhere (10). Inoculation was accomplished by mixing equal parts of the inoculum and 2 per cent cerelose solution just before needed and adding 100 ml. of this mixture to each pot. The inoculum was then mixed into the sand and the sand leveled. Each control pot received 100 ml. of the 2 per cent cerelose solution. Seeds of the parental varieties were planted in both inoculated and control pots. All seeds were planted to a depth of about 1 in.

Inoculation tests for near-wilt reaction were made in metal pans by the clipping method (10). Sand temperature was maintained close to 21° C. in Wisconsin soil-temperature-control tanks. Between 12 and 20 days after inoculation, notes were taken on disease development and the plants assigned to one of the following classes: healthy, slightly diseased, moderately diseased, severely diseased, and dead. From these notes it was possible to determine the genotypes of the lines being tested.

Determinations of linkage intensities were made from backcross data by the product method using table 1 of Immer and Henderson (2), from F_1 data by the method of maximum likelihood (4), and from combinations of F_3 data from different crosses and F_3 and backcross data from the two crosses by Fisher's scoring method as outlined by Kramer and Burnham (4). All observations were made in the coupling phase except those involving internode length.

EXPERIMENTAL RESULTS

Characteristics of the three varieties serving as parents in this study

TABLE 1.—*Characteristics of seedlings of Delwiche Commando, Davis Perfection, and Thomas Laxton grown in the greenhouse*

Variety	Reaction to		Av.[a] no. nodes	Plant[a] height, inches	Av.[a] length of internode	Length[a] of longest internode
	Wilt	Near wilt				
Delwiche Commando	Res.	Res.	6.5	4.2	0.7	0.9
Davis Perfection	Susc.	Susc.	6.0	4.4	0.7	1.0
Thomas Laxton	Susc.	Susc.	6.0	9.5	1.6	2.8

[a] Based on measurements of 10 seedlings examined 15 days after planting.

are indicated in table 1. Delwiche Commando was resistant and the other two varieties were susceptible to the two diseases as previously mentioned. Thomas Laxton was long vined and the other two varieties were short-vined. It is clear from the table that the distinction between tallness and dwarfness in this study was attributable to length of internode rather than to number of internodes. The internode length factor pair has been designated Le le after White (11). Wade (9) designated the factor pair for reaction to Fusarium wilt as Fw fw. A convenient genetic designation was needed for the factor pair governing reaction to near-wilt and the symbols Fnw fnw were adopted.

In table 2 are summarized the ratios for height and for reaction to wilt and near-wilt in the two crosses studied.

TABLE 2.—*Distributions and frequencies of lines from crosses of Delwiche Commando with Thomas Laxton and Davis Perfection for reaction to wilt, reaction to near-wilt, and internode length; and P values from* X^2 *for goodness-of-fit*

Cross[a]	Generation	Character	Factor pair		Number of lines			Total	Range of P
			X	x	XX	Xx	xx		
TL × DC	F_3	Wilt reaction	Fw	fw	32	36	17	85	< 0.01
Do	do	Near-wilt reaction	Fnw	fnw	49	32	4	85	< 0.01
Do	do	Internode length	Le	le	28	55	28	111	0.70–0.50
Do	F_2, BC_1	Wilt reaction	Fw	fw	79	87		166	0.50–0.95
Do	do	Near-wilt reaction	Fnw	fnw	87	79		166	0.50–0.95
Do	do	Internode length	Le	le		94	77	171	0.20–0.10
DP × DC	F_3	Wilt reaction	Fw	fw	42	87	29	158	0.20–0.10
Do	do	Near-wilt reaction	Fnw	fnw	48	77	33	158	0.30–0.20
Do	F_2, BC_1	Wilt reaction	Fw	fw	15	23		38	0.20–0.10
Do	do	Near wilt reaction	Fnw	fnw	15	23		38	0.20–0.10
Both	F_3	Wilt reaction	Fw	fw	74	123	46	243	0.05–0.01
Do	do	Near wilt reaction	Fnw	fnw	97	109	37	243	< 0.01
Do	F_2, BC_1	Wilt reaction	Fw	fw	94	110		204	0.30–0.20
Do	do	Near wilt reaction	Fnw	fnw	102	102		204	> 0.99

[a] TL = Thomas Laxton; DC = Delwiche Commando; DP = Davis Perfection.

In the F_3 of Thomas Laxton × Delwiche Commando, ratios for reaction to wilt and near-wilt were not in agreement with the $1:2:1$ ratio. Apparently the conditions provided in this test were not optimum for the expression of wilt and near-wilt, since there were marked negative deviations from the expected numbers of lines in the segregating and homozygous susceptible classes. The observed ratio for internode length of $28:55:28$ was a close fit to a $1:2:1$ ratio. In the F_2 of the first backcross generation, ratios obtained for reaction to wilt and near-wilt were in satisfactory agreement with the expected $1:1$ ratios. The ratio for internode length also was a satisfactory fit to a $1:1$ ratio, P being between 0.20–0.10.

In the F_3 of the Davis Perfection × Delwiche Commando cross, the ratios obtained for reaction to wilt and near-wilt were satisfactorily explained on the basis of a $1:2:1$ segregation though there was a deficiency in both ratios for homozygous susceptible lines. Ratios for reaction to wilt and near-wilt in the F_2 of the first backcross generation in this cross were satisfactory when compared to $1:1$ ratios, though there was a deficiency in both for homozygous resistant lines.

When F_3 ratios for disease reaction in the two crosses were combined, ratios were obtained which were not satisfactorily explained on the basis of single factor segregations. The deficiencies for the susceptible reaction were responsible for this failure of agreement with $1:2:1$ ratios. Good

TABLE 3.—*Associations between reaction to wilt, reaction to near-wilt, and internode length in crosses of Delwiche Commando with Thomas Laxton and Davis Perfection varieties*

Cross[a]	Factor pairs				Generation	linkage phase	No. lines in these classes									T	P from X² for independence	Recombination percentage
							XX YY	XX Yy	Xx YY	Xx Yy	xx YY	xx Yy	XX yy	Xx yy	xx yy			
TL×DC	Fw	fw	Fnw	fnw	F₃	C	20	9	16	19	13	4	3	1	0	85	0.10–0.05	54.9 ± 7.5
Do	do	do	do	do	BC	C	48	31	39	48						166	0.05–0.01	42.2 ± 3.8
DP×DC	do	do	do	do	F₃	C	8	20	29	45	11	12	14	13	6	158	0.10–0.15	61.2 ± 4.6
Do	do	do	do	do	BC	C	9	6	6	17						38	0.05–0.01	31.6 ± 7.6
Both	do	do	do	do	F₃	C	28	29	45	64	24	16	17	14	6	243	0.10–0.05	54.4 ± 4.2
Do	do	do	do	do	BC	C	57	37	45	65						204	< 0.01	40.2 ± 3.4
TL×DC	Fw	fw	Le	le	F₃	R	5	17	10	30	13	8	20	6	2	111	< 0.01	28.2 ± 4.1
Do	do	do	do	do	BC	R		36	58				45	32		171	< 0.01	40.0 ± 3.4
Both					F₃	BC												35.1 ± 2.9
TL×DC	Fnw	fnw	Le	le	F₃	R	14	26	11	19	2	2	15	6	3	98	0.70–0.50	47.6 ± 6.9
Do	do	do	do	do	BC	R		47	50				45	31		173	0.20–0.10	45.1 ± 3.8

[a] TL = Thomas Laxton; DC = Delwiche Commando; DP = Davis Perfection.

agreement with the expected 1:1 ratios was obtained, however, for the combined ratios from backcross data. Since the detection and estimation of linkage can only be accurate when the single factor segregations are good, it is clear that more reliable linkage determinations can be obtained in this study from the backcross than from the F_3 data.

Data are summarized in table 3 for associations between internode length and reaction to wilt and near-wilt.

Recombination percentages from F_3 data for the comparison Fw-Fnw were 54.9 ± 7.5 per cent for the Thomas Laxton × Delwiche Commando cross, 61.2 ± 4.6 per cent for the Davis Perfection × Delwiche Commando cross, and 54.4 ± 4.2 per cent for the combined sources of information from F_3 data. These data suggest independence of the Fw-Fnw loci. The value of this information from F_3 data was reduced, however, by the poor single factor ratios involved. Recombination percentages calculated from backcross data were 42.2 ± 3.8 per cent for the Thomas Laxton × Delwiche Commando cross, 31.6 ± 7.6 per cent for the Davis Perfection × Delwiche Commando cross, and 40.2 ± 3.4 per cent for the combined sources of information from backcross data. These data suggest that the Fw-Fnw loci are in the same linkage group though rather far apart. Since the backcross data were in good agreement with expectation, it seems reasonable to conclude that the evidence for linkage of the Fw-Fnw loci is greater than that against such a linkage.

Segregation for internode length occurred in only the Delwiche Commando × Thomas Laxton cross in which F_3 data showed a recombination value of 28.2 ± 4.1 per cent for the comparison Fw-Le and backcross data gave a value of 40.0 ± 3.4 per cent. The combined F_3 and backcross data gave a recombination of 35.1 ± 2.9 per cent between the Fw and Le loci. This value is in agreement with that of about 31 per cent reported by Wade (9). When a linkage calculation is made by the product method from the data reported by Wade, a value of 32.0 ± 1.8 per cent is obtained which is reasonably close to the recombination percentage found in this study.

For the association Fnw-Le in the Thomas Laxton × Delwiche Commando cross, recombination percentages were obtained of 47.6 ± 6.9 per cent from F_3 data and 45.1 ± 3.8 per cent from backcross data. Neither of the P values calculated from χ^2 for independence from these two sources of information was significant and with the high recombination percentages suggested independence of the Fnw and Le loci. This information adds little to what has been discussed earlier concerning the Fw-Fnw relationship. If the loci for reaction to wilt and near-wilt are in the same linkage group, then clearly the Fnw locus does not lie between those of Fw and Le.

DISCUSSION AND SUMMARY

The object of this investigation has been to determine the linkage relations of some genes governing reaction to the wilt and near-wilt diseases of peas. Evidence has been presented suggesting that these factors are

associated with a loose linkage of 40.2 ± 3.4 per cent as determined from backcross data from the two crosses studied. The F_3 data indicated independence of the two factors, but this information was shown to be less reliable than the backcross results.

In breeding programs devoted to the production of new varieties resistant to near-wilt, parental stocks have been used for crossing with the wilt- and near-wilt-resistant Delwiche Commando, or with related selections, that are either wilt-resistant or are susceptible to both wilt and near-wilt. In the former type of cross, *i.e.*, between varieties resistant to wilt but differing in reaction to near-wilt, the presence or absence of any degree of linkage between Fw and Fnw would have no significance. Many of the crosses that have been made are of this type. In the latter type of cross, *i.e.*, between a variety susceptible to both wilt and near-wilt and one resistant to the two diseases, both factors for resistance would enter the cross from the same parent and in the event of close linkage a larger proportion of the progenies than expected on the basis of independence would be resistant to both diseases. This favorable situation will not occur in breeding for wilt and near-wilt resistance because of the loose linkage suggested by the results of this investigation.

The symbols Fnw fnw have been suggested for designation of the factor pair governing reaction to the near-wilt disease.

Fw was found to be associated with the factor for internode length, Le, in combined F_3 and backcross data with 35.1 ± 2.9 per cent of recombination, confirming the findings of Wade (9) in 1929. Fnw appeared to be inherited independently of the Le locus. If Fw and Fnw are on the same chromosome, Fnw does not lie between Fw and Le but to one side or the other of this gene pair.

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FUSARIUM STEM CANKER AND WILT· OF DELPHINIUM[1, 2]

Thomas Laskaris[3]

Occasional reports have appeared of the occurrence in the United States of two little-known delphinium diseases, but the causal relationship of *Fusarium* to these diseases has not been established nor have the species involved been identified.

Fusarium wilt has been reported from Ohio (5, p. 47), and two reports from Florida (6, p. 440, and 7, p. 350) mention a severe wilt of delphinium in flower beds in Duval and Ocala counties. A root rot reported as common in southern Arizona also has been attributed to an undetermined species of *Fusarium* (8, p. 263). Leonian (3) describes very briefly a Fusarium stem canker reported from Delaware, Florida, and West Virginia. Leonian was of the opinion that the disease undoubtedly occurs in other States and he informed the writer that it was especially prevalent and severe in the summer of 1940 in West Virginia.

The occurrence of Fusarium stem canker in Connecticut, New Jersey, and New York was reported by the writer (1, 2). A typical cankered stem is shown in figure 1. Commonly associated with the canker, and not de-

Fig. 1. Stem canker of delphinium.

scribed by Leonian, are the numerous salmon-colored sporodochia on the surface of the cankered area. When cankered stems were split, abundant white mycelium and spores of *Fusarium* were found. The disease often spread to the crown and killed the affected plant.

The writer also reported a Fusarium wilt of delphinium occurring in epidemic proportions in Connecticut (2). In this instance, the grower at-

tributed the death of the plants to a deficiency in the soil. The disease was restricted to a certain area of a large planting where it had caused similar losses in previous years. The grower also had observed that seedlings very frequently became diseased, often in the same season, if they were set in the same places where other plants had succumbed to the disease. The symptoms noted at the time by the writer were a progressive yellowing of the foliage beginning with the basal leaves and, finally, a wilting of the entire plant. There was little evidence of root rot, but a brown vascular discoloration was evident in cut portions of the crown, the roots, and young shoots at the base of the stem.

The canker was observed only on commercial garden hybrids, whereas the wilt organism was isolated from *Delphinium cardinale* and *D. consolida* as well as from the hybrids from Connecticut.

In the present study the writer ascertained more thoroughly the disease symptoms and studied the relation of isolates of *Fusarium* to the stem canker and the wilt. The pathogenicity and the identity of the isolates were determined.

SYMPTOMS

On plants sprayed with a spore suspension of the *Fusarium* isolated from stem cankers, the first disease symptoms are small brown to brownish-gray spots on the stems. These spots gradually enlarge and several may coalesce to form one or more large spots. Brown sunken watersoaked lesions may appear on the petioles and spread to the stem. The lesions enlarge both laterally and longitudinally, but the longitudinal spread is the more rapid. The cankered area is chocolate-brown and characteristically moist or watersoaked. On large plants, cankers may be over a foot long. In the advanced stages of development of a canker the diseased tissue in the center of a canker may rupture. Eventually the tissue in the central part of the canker shrinks or collapses while the thin epidermal layer remains intact and appears white or bleached.

Numerous salmon-colored sporodochia of the *Fusarium* are distributed irregularly over the bleached area. They bear large numbers of predominantly 3-septate macrospores (Fig. 2, C, D). The pith cavity of a cankered stem is almost completely filled with white mycelium bearing numerous 0-septate and 1-septate spores. In time the fungus reaches the crown, where it causes a local necrosis. After passing into the crown the fungus invades and discolors the vascular tissues, and the young shoots commonly present at the base of a plant soon wilt. In late stages of disease the parts of the stem above the canker may wilt.

The symptoms of Fusarium wilt may, at first glance, be confused with the wilt symptoms due to *Sclerotium delphinii* and *S. rolfsii*. However, the characteristic sclerotia and mycelium of *Sclerotium* are sufficient to distinguish that wilt from Fusarium wilt. When a mature plant with symptoms of Fusarium wilt is cut, a typical brown vascular discoloration is evident,

as in stem canker. The discoloration may extend from the crown into the main roots and for some distance upwards into the main stem and young shoots. Yellowing of the leaves and a wilting and drooping of the inflorescence may occur.
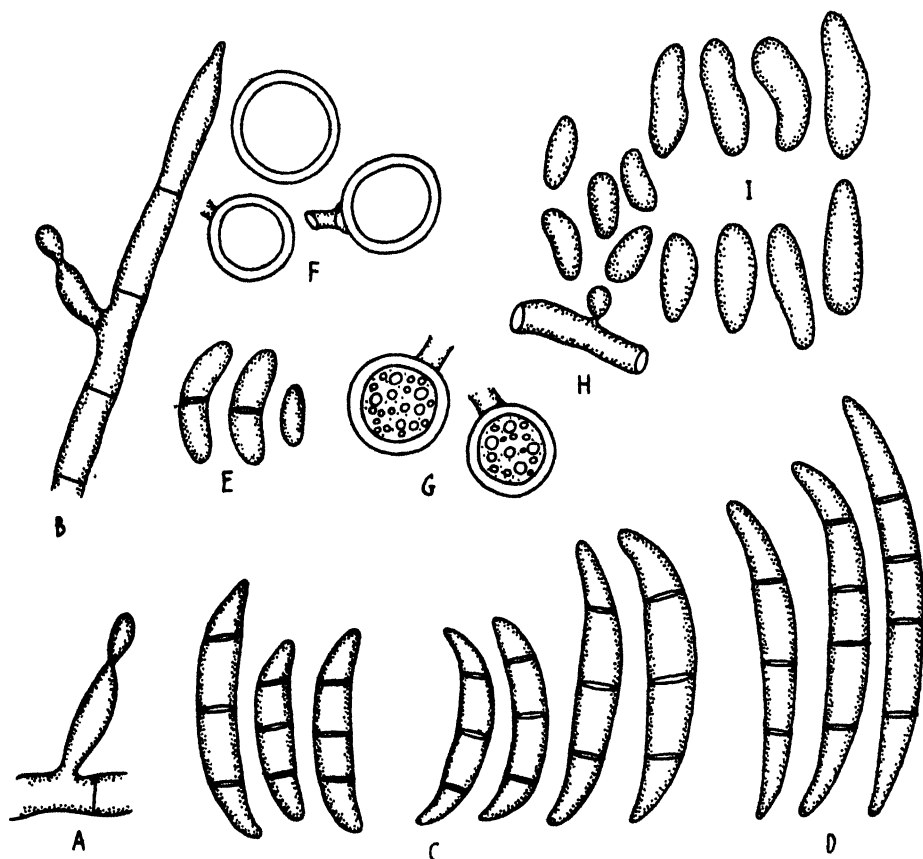
FIG. 2. *Fusarium oxysporum* f. *delphinii*. A, B. Conidiophores from sporodochia on stem. C, D, E. Conidia from sporodochia on stem. F, G. Chlamydospores from 72-day-old potato-dextrose agar culture. H, I. Conidiophore and conidia from 72-day-old potato-dextrose agar culture.

IDENTITY OF THE FUSARIUM ISOLATES ASSOCIATED WITH
DELPHINIUM STEM CANKER AND WILT

There was a marked similarity among the more than forty isolates of *Fusarium* from plants affected with stem canker and wilt disease. They were characterized by the pink to vinaceous coloration produced on potato-dextrose agar. They belonged to the section Elegans of the genus. Representative isolates from stem canker and wilt were sent to Dr. W. C. Snyder who corroborated the writer's determinations and further identified the isolates as belonging to the species group *Fusarium oxysporum* (Schl.) Sny. and Han. Snyder and Hansen (4) have shown, from a study of single-spore

cultures, that the present basis for division of the section Elegans into the three subsections Orthocera, Constrictum, and Oxysporum is untenable and invalidates the species in these sections. Thus the isolates from delphinium identified by Snyder as *Fusarium oxysporum* (Schl.) Sny. and Han. might, as they now appear in culture, be called *F. orthoceras* or possibly *F. bulbigenum* by Wollenweber and Reinking; but they might classify the sporodochial stage on the host as *F. oxysporum* (Schl.).

According to the species concept as proposed by Snyder and Hansen, all members of section Elegans constitute one natural species group which on the bases of usage and priority may be called *Fusarium oxysporum*. The numerous parasitic forms are recognized principally by their selective pathogenicities, and those that cause vascular wilts are merely biologic forms of one and the same species.

TAXONOMY AND MORPHOLOGY

The inoculation tests demonstrated that Fusarium isolates from both the stem canker and the wilt are true vascular parasites, highly pathogenic and capable of inducing a typical wilt. While it was not demonstrated that the wilt isolates are capable of inducing a stem canker, the fact that the stem canker isolates caused a severe wilt when introduced into the soil around healthy greenhouse and field plants indicates that only one species of *Fusarium* is involved. Furthermore, many of the isolates from both diseases were indistinguishable morphologically. Since the parasitic forms of *Fusarium oxysporum* are highly specialized as to host and since there has been no previous description of a species of *Fusarium* pathogenic to delphinium, the writer feels justified in describing, as a new parasitic form of *Fusarium oxysporum*, the isolates pathogenic to delphinium. It is proposed therefore that the organism causing delphinium stem canker and delphinium wilt be named as follows:

Fusarium oxysporum (Schl.) Sny. and Han. f. delphinii n.f.[4]

Microconidia numerosissima, plerumque aseptata. Macroconidia ambobus finibus gradatim attenuata, prope in medio cylindrica, leviter pedicellata. et penitus aequabiliter plerumque curvata, regulariter 3-septata 32.6 × 3.4 (23.1–42 9 × 3.0–4.3) μ, 4–5 septata conidia rara. Mycelium aerium plerumque robustum, album; substratum, in agare dextroso opulenta Solani tuberosi, primo incoloratis tunc rosaceum aut vinaceum; in culturis nulla sporodochia aut sclerotia; sporodochia magna salmonea plerumque 3-septatis conidia hospite producta. Hab. in decoloratis fibrovascularibus fascibus et in caulibus delphinii.

Microconidia very numerous, dominantly aseptate. Macroconidia gradually attenuated toward both ends, nearly cylindrical in the middle, somewhat pedicellate, and usually uniformly curved throughout, typically 3-sep-

tate, 32.6 × 3.4 (23.1–42.9 × 3.0–4.3) μ.   Four- and 5-septate conidia are rare. Aerial mycelium usually well developed, white; substratum, on potato agar rich in dextrose, colorless at first then light pinkish to vinaceous; no sporodochia or sclerotia in culture; large salmon-colored sporodochia containing mostly 3-septate macrospores produced on the host.   Habitat: In discolored fibrovascular bundles of delphinium and on stems of delphinium.

Type locality: Ridgefield, Connecticut.

Type specimens deposited in Herbarium of the New York Botanical Garden.

The morphological characters of the organism are shown in figure 2.

Conidia from aerial mycelium close to the substratum of culture medium and conidia from a large sporodochium on delphinium stem were measured:

From 53-day-old culture on potato plug:

0-septate (99 per cent), 6.7 × 2.3 μ

1-septate (rare, 1 measured), 13.0 × 2.9 μ

From 62-day-old culture on hard carrot agar plus 2 per cent dextrose:

0-septate (99 per cent), 8.7 × 2.6 μ

1-septate (0.8 per cent), 13.1 × 3.5 μ

2-septate (rare, 1 measured), 20.8 × 3.6 μ

From delphinium stem:

0-septate (rare, 2 measured), 15.8 × 3.1 μ

1-septate (8 per cent), 19.2 × 3.3 μ

2-septate (1 per cent), 24 3 × 3.3 μ

3-septate (90 per cent), 32.8 × 3.7 μ

4 septate (rare, 1 measured), 33.3 × 3.3 μ

Chlamydospores from a 53-day-old culture on potato plug were terminal or intercalary, without septa, and measured 8.6 × 7.0 μ.

PATHOGENICITY

The pathogenicity of a number of stem canker isolates was demonstrated in both greenhouse and field experiments.   The same isolates were capable of producing a typical wilt disease in addition to the stem canker.

In two representative greenhouse tests, the virulence of a stem canker isolate was tested on 20 hybrid delphinium seedlings growing in 3-in. pots. Inoculum was prepared by growing the fungus isolate for 1 month in an autoclaved mixture of sand and cornmeal.   The top inch of soil from each pot was replaced by inoculum, and initial wilt symptoms appeared on the seedlings in 20 days.   Inoculated plants were retarded in growth and stunted (Fig. 3).   Leaves yellowed slightly, but the marked yellowing usually associated with disease in field plants did not appear.   The leaves sooner or later collapsed and began to turn brown, and the petioles began to bend.   Once the initial symptoms of wilt appeared, the affected plants succumbed rapidly, usually within 4 to 5 days.   All of the 20 inoculated plants became diseased, while 10 control plants that had received sterile sand-cornmeal mixture in-

stead of the inoculum failed to become diseased in any way during the same period. The fungus used for inoculation was in all cases easily reisolated from the diseased crowns and roots of the affected plants.

The ability of the stem canker isolates to cause a wilt was further proved in field experiments. Two beds containing 40 and 20 seedlings respectively were inoculated with two stem canker isolates. The inoculum was prepared by growing the fungi in quart jars containing a steamed sand-cornmeal mixture. The soil around the base of each plant was removed and replaced with inoculum. The amount of inoculum applied in this way was very small, yet 22, or approximately one-third, of the inoculated plants became

Fig. 3. Plant at left is healthy (check); plant at right, inoculated with a Fusarium stem canker isolate, is wilting.

diseased during the first growing season. In a typical diseased plant the leaves showed signs of collapse and their edges turned inward. The leaves near the tip of the young inflorescence turned yellow first and later more of the older leaves yellowed. A brown discoloration of the vascular tissues was seen in the crowns of all diseased plants. A few of the plants developed stem canker symptoms. In these plants grayish-brown lesions developed from the base of the stem upwards, and the interior of such stems contained abundant mycelium of the fungus that had been used for inoculation.

These isolates also were tested for their ability to produce the stem canker disease. Spore suspensions were prepared by diluting with water the liquid cultures on which the isolates had been grown. A bed of 50 plants was sprayed with a mixed spore suspension of the two isolates used in the field tests. A cheesecloth tent was kept over the inoculated plants for 5 days.

The symptoms of stem canker did not appear until about 3 weeks after the plants were inoculated. Seventeen of the 50 plants inoculated developed symptoms of stem canker.

The two wilt isolates from Connecticut were highly pathogenic in greenhouse tests, 18 of 20 inoculated plants becoming diseased. In general the symptoms were identical with those caused by the stem canker isolates in similar greenhouse tests.

CONTROL

Leonian (3) recommends that plants diseased by Fusarium stem canker be sprayed with Bordeaux and that the spikes be cut off several inches below the canker before the fungus infects the crown. However, the fact that the fungus may cause a wilt suggests that complete control will necessitate the adoption of soil disinfection methods wherever the disease has become well established. No attempt has been made to work out a method of control, since the efforts of the writer have been confined to a study of the identity and pathogenicity of the isolates of *Fusarium* associated with these diseases.

SUMMARY

The pathogenicity of Fusarium isolates associated with the stem canker and wilt diseases of delphinium was ascertained in both greenhouse and field experiments, and the disease symptoms were described in detail. The isolates appear to be true vascular parasites and are highly pathogenic. The name *Fusarium oxysporum* (Schl.) Sny. and Han. f. *delphinii* n. f. is proposed for the causal organism of the stem canker and wilt diseases of delphinium.

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FLOODING AS A MEANS OF DESTROYING THE SCLEROTIA OF SCLEROTINIA SCLEROTIORUM

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INTRODUCTION

The sclerotiniose disease (caused by *Sclerotinia sclerotiorum* (Lib.) D By.) of various vegetable crops has become of increasing importance to growers in many sections of the United States during recent years, causing losses in varying degree both in the field and during transit to market.[2] For a long time it has been of importance in celery and lettuce but during the past ten years has become a serious problem in tomatoes, potatoes, beans, peppers, and other vegetables. While the disease is widely distributed over the country, one of the most heavily infected areas at this time is in south Florida, where since 1938 it has appeared in practically all of the vegetable-growing areas of the lower part of the peninsula and has become a factor in the production of several crops.

Although the sclerotiniose fungus has been known for many years, attempts to control it have been limited to one or two crops and to fairly restricted conditions of culture, such as small fields, greenhouses, and seedbeds.[3] One of the most comprehensive attacks on the problem was by Brooks[4] who showed that a combination of flooding and soil treatment with cyanamid would kill a large percentage of the sclerotia in muck soils. His flooding work has suggested a method of control that may be adapted to many areas of Florida and other States where ample supplies of water are readily available. In order to study the effect of flooding on sclerotial decay in greater detail, a series of experiments was conducted in south Florida from 1945 through 1947, the results of which are presented in this paper.

SOIL TYPES AND DRAINAGE CONDITIONS

Farm lands in south Florida are classed under three large soil types—marl, muck, and sand—which vary widely in humus content, texture, and water-holding capacity. The sandy soils along the east coast are low in humus, are well drained by an elaborate system of canals, and are subject to wide fluctuations in moisture. Sub-irrigation is used in many instances;

thus some fields may remain fairly wet while others are relatively dry. When temperatures are below 70° F., disease incidence under these conditions varies in intensity from very light to very severe, depending upon soil moisture from both rain and irrigation.

The marl soils south of Miami are fine in texture and have a high water-holding capacity. Fields in this area are moist at all times, particularly so during the winter months when hours of sunlight are fewer than in other seasons and rates of soil moisture evaporation are low. The water table is seldom more than 3 to 4 feet below the soil surface and drainage is fairly slow; consequently, moisture conditions favor disease infection during much of the growing season.

The muck soils in the Everglades are composed largely of organic matter and have a high water-holding capacity, but because of their loose friable condition in cultivated fields they do not maintain so high a degree of soil moisture as do the marl soils. They are, however, sufficiently wet during most of the growing season to make sclerotiniose infection possible.

With the variations in soils and their water-holding capacities, considerable differences might be expected in the persistence of the sclerotiniose fungus in the fields from year to year. Some of the areas are subject to intermittent flooding by rain during the summer, while others can be flooded only by pumping water from near-by canals. There are also large areas that cannot be flooded by any means available at the present time.

PROCEDURE

The laboratory investigations consisted in placing 6 in. of marl, muck, and sandy soils in metal containers, each soil type being replicated three to five times. Sheets of plastic wire screen were cut to fit the containers; the sclerotia were inserted between two of these and placed on top of the soil. Tap water was kept at a level approximately 2 in. above the surface. Examinations were made at regular intervals and the sclerotia that had not decayed were counted.

Field tests were made with flats measuring $12 \times 12 \times 3$ in., with plastic screen bottoms. The sclerotia were distributed uniformly on the screen in the flats and a second sheet of screen was placed over them, after which the soil level was adjusted to the desired depth. These were located at different points in the fields. Inspections were made by removing the layer of soil and the top sheet of screen, which left the sclerotia in their original position.

RESULTS OF LABORATORY TESTS

Data for the laboratory tests conducted in 1945 and 1946 are shown in table 1. Separate containers were prepared for each sampling for each soil to insure that none was disturbed until time for making the single reading of results. In one test 92 per cent of the sclerotia were decayed in the marl soils in 30 days; in the other, 100 per cent were decayed in 24 days. Decay in the muck soils was 100 per cent in 23 days in one test and the same amount

in 31 days in the second. In the sandy soils there was 100 per cent decay in 23 days in one test and 86 per cent decay in 31 days in the other. Apparently the amount of decay was somewhat higher in the muck soils than in either the marls or sands, but the rate of decay was not appreciably faster.

Previous field observations indicated that under normal weathering

TABLE 1.—*Rates of sclerotial decay when subjected to flooding over marl, muck, and sandy soils for the periods shown. Fifty sclerotia per treatment in all cases. Sclerotia placed on soil surface and flooded to depth of 2 in. in metal containers*

Date of reading	Number of decayed sclerotia			Mean temperature (°F.)
	Marl	Muck	Sand	
Apr. 28, 1945	0	0	0	78.2
May 8	2	22	16	72.6
May 21	40	50	50	77.4
May 28	46			76.0
Percentage decayed	92	100	100	
Sept. 27, 1946	0	0	0	81.0
Oct. 14	45	42	17	78.4
Oct. 21	50	49	31	76.7
Oct. 28		50	43	76.0
Percentage decayed	100	100	86	

sclerotia on the surface of the soil decayed somewhat faster than those buried 2 and 3 in. deep. Since sclerotia are found in commercial fields at various depths in the soil, tests were conducted in 1947 to study the rates of decay during flooding when sclerotia were placed on the surface and at a depth of 2 in. Four containers with 25 sclerotia each were used for each

TABLE 2.—*Rates of sclerotial decay at two levels in marl, muck, and sandy soils when subjected to flooding for the periods shown. One hundred sclerotia per treatment in all cases*

Date of reading	Number of decayed sclerotia						Mean temperature (°F.)
	Marl		Muck		Sand		
	Surface	2 in. deep	Surface	2 in. deep	Surface	2 in. deep	
Oct. 1, 1947	0	0	0	0	0	0	76.0
Oct. 16	30	40	69	68	71	15	75.9
Oct. 24	67	69	94	100	97	44	79.0
Nov. 3	99	86	100		100	76	78.2
Nov. 14	100	100				100	75.9
Percentage decayed	100	100	100	100	100	100	
Nov. 14	0	0	0	0	0	0	75.9
Nov. 28	93	96	87	74	76	31	77.2
Dec. 8	100	100	100	100	100	100	71.1
Percentage decayed	100	100	100	100	100	100	

planting and all soils were flooded to a depth of 2 in. above the surface. The data for two tests (Table 2) indicate that rates of sclerotial decay were not appreciably slower at the 2-in. depth than on the surface. Although rates of decay were not consistent for soil types or for depth of planting, 100 per cent decay occurred in all cases in 24 to 45 days. A possible explanation of this wide variation in time may have been the fluctuation of the soil microflora, other tests having shown that saprophytic fungi and bacteria are closely associated with sclerotial decay.

Since some areas in south Florida are flooded for short periods during the summer by rains and other areas can be flooded only by pumping, tests were conducted in 1947 to determine the effect of intermittent vs. continuous flooding. Containers with sclerotia on the soil surface were prepared as described above, one set being flooded continuously and the other at intervals of 3 days. The interval flooding held the soils at the saturation level at all times and permitted aeration of the surface half of the time. The flood level was 2 in. above the soil surface in all cases. Data for the two tests (Table 3) indicate that interval flooding was almost as effective in

TABLE 3 —*Rates of sclerotial decay on marl, muck, and sandy soils when flooded continuously and at 3 day intervals for the periods shown. One hundred sclerotia per treatment in all cases*

| Date of reading | Number of decayed sclerotia | | | | | | Mean temper ature (°F.) |
| | Marl | | Muck | | Sand | | |
	Continuous	Intermittent	Continuous	Intermittent	Continuous	Intermittent	
Sept. 12, 1947	0	0	0	0	0	0	81.0
Sept. 26	47	7	85	94	84	88	80.0
Oct. 2	71	39	100	100	100	100	76 6
Oct. 13	94	78					75 6
Oct. 23	100	98					78 1
Percentage decayed	100	98	100	100	100	100	
Oct. 23	0	0	0	0	0	0	78.1
Nov. 3	55	50	57	23	42	34	78.2
Nov. 10	95	90	98	73	89	84	75.1
Nov. 17	100	96	100	94	100	94	78.0
Percentage decayed	100	96	100	94	100	94	

destroying sclerotia as continuous flooding. The number of days required for 100 per cent decay was approximately the same for intermittent as for continuous flooding, indicating that the element of time was more important than that of keeping the sclerotia submerged continuously.

Most agricultural lands in south Florida stand idle during the summer,

being planted with a cover crop, such as Sesbania, or allowed to grow up in weeds and grasses. This provides much needed shade and adds appreciable amounts of organic matter to the soils. Since the latter is a part of the normal medium for soil microflora and since the microflora are associated with sclerotial decay, a study was made of the effect of green organic matter on decay when used in conjunction with flooding. Since preliminary tests with green organic matter had given conflicting results in rates of sclerotial decay, two tests were conducted in 1947 in which 2 in. of green organic matter was added to sets of containers of marl, muck, and sandy soils, while equal numbers of containers were held without the organic matter. Sclerotia

TABLE 4.—*The effect of green organic matter on sclerotial decay in marl, muck, and sandy soils when subjected to flooding for the periods shown. One hundred sclerotia per treatment in all cases*

Date of reading	Number of decayed sclerotia						Mean temperature (°F.)
	Marl		Muck		Sand		
	Green organic matter	None	Green organic matter	None	Green organic matter	None	
Nov. 20, 1947	0	0	0	0	0	0	76.0
Dec. 2	61	51	49	52	33	56	73.1
Dec. 12	94	91	86	92	68	93	74 3
Dec. 18	100	100	100	96	91	100	75.5
Percentage decayed	100	100	100	96	91	100	
Dec. 18	0	0	0	0	0	0	75.5
Dec. 29	41	40	53	37	38	40	65 6
Jan. 9, 1948	85	47	92	86	88	93	66.5
Jan. 20	100	100	100	100	100	100	62.2
Percentage decayed	100	100	100	100	100	100	

were placed between sheets of screen at the soil surface level in all cases and the green organic matter was added on top of the screens. Flooding to a depth of 2 in. above the soil surface was continuous and readings were made at approximately 10-day intervals.

The addition of green organic matter in this manner did not materially influence the rate of sclerotial decay in any case (Table 4). As in previous tests, total decay occurred within 25 to 40 days.

FIELD FLOODING

Tests were conducted in 1946 and 1947 to study the rates of sclerotial decay in commercial fields following periods of continuous flooding. Flats measuring $12 \times 12 \times 3$ in. were placed on the soil surface and at depths of 2 in., each containing 25 sclerotia, four flats being used for each treatment. The water level was held at a depth of 1 to 2 ft. over the fields throughout the tests. Readings were made as soon as the water receded to a point where the flats could be removed.

The rates of decay were fairly uniform in all cases (Table 5) and the time required for total or near total decay was approximately the same as reported in laboratory tests. These tests were repeated in 1947, but owing to excessive summer rains the fields were flooded for about 8 weeks and

TABLE 5.—*Rates of sclerotial decay at two levels in a cultivated field following flooding. One hundred sclerotia per treatment in all cases. Flooding periods, May 16 to June 12, 1946, and Aug. 25 to Sept. 15, 1946*

Date of reading	Number of decayed sclerotia		Date of reading	Number of decayed sclerotia	
	Surface	2 in. deep		Surface	2 in. deep
May 17, 1946	0	0	Aug. 20, 1946	0	0
June 10	95	88	Sept. 23	99	93
June 17	100	97	Percentage		
Percentage decayed	100	97	decayed	99	93

accurate readings could not be made. Flats were placed in the fields on June 15, and when removed on September 20 all sclerotia were decayed.

Three tests were conducted in 1946 in which flats containing 25 sclerotia were placed on the soil surface and at depths of 2 and 3 in. respectively in order to study the rates of sclerotial decay under conditions of natural rainfall. Two tests were made on sandy soil and one on marl soil, thus affording wide variations in soil moisture in one case and fairly constant moistures in the other.

Data for these tests (Table 6) indicate that 100 per cent decay occurred

TABLE 6.—*Rates of sclerotial decay in cultivated fields when subjected only to the prevailing rainfall. Twenty five sclerotia per treatment in all cases*

Date of reading	Number of decayed sclerotia			Accumulated rainfall (inches)
	Surface	2 in. deep	3 in. deep	
June 5, 1946	0[a]	0	0	0.00
Aug. 5	0	5	12	10.87
Sept. 9	5	6	12	21.57
Sept. 23	10	6	12	27.86
Percentage decayed	40	24	48	
June 5	0[a]	0	0	0 00
Aug. 7	25	4	6	10.87
Sept. 17	25	25	25	27.56[b]
Percentage decayed	100	100	100	
Apr. 1	0[c]	0	0	0.00
June 14	3	0	0	15 42
July 30	7	10	0	27.51
Oct. 22	24	11	1	48.40
Percentage decayed	96	44	4	

[a] Sandy soil.
[b] Field flooded for approximately 6 weeks, July 1 to Aug. 15, 1946.
[c] Marl soil.

only where one field was flooded for approximately 6 weeks. A fairly high degree of decay, apparently due to fungi and bacteria, occurred at the surface level on the marl soils, but appreciably less decay occurred at the 2-in. and 3-in. levels. Decay on the sandy soils was moderate at all levels but not sufficiently high to be of importance in disease control. Since rainfall during the course of these tests was average for the particular time of year, it is apparent that natural moisture conditions are not likely to be sufficiently high to cause appreciable decay of sclerotia in most years or locations.

DISCUSSION

When marl, muck, and sandy soils containing sclerotia of *Sclerotinia sclerotiorum* were flooded, complete sclerotial decay occurred within 23 to 45 days. Rates of decay were not consistent among either soil types or tests, being rapid in some instances and slow in others. However, near-total to' total decay occurred in all cases under prolonged flooding. Rates of decay were about the same for sclerotia that were buried at different depths in the soil. When alternate floodings and drainings were made at intervals of 3 days, rates of sclerotial decay were approximately as high as when flooding was continuous. Presumably decay was influenced to a marked degree by various soil microflora in both instances, and the 3-day flooding period appeared to be ample for support of their activity. Under the conditions of the tests reported, addition of green organic matter to soil surface did not influence rates of sclerotial decay. The importance of such material in its relation to soil microflora, however, should not be discounted. The types of organic material, their state of decomposition, rate of application, and especially degree of mixture with the soil might well play an important part in the decay of sclerotia. When sclerotia were placed in commercial fields and subjected to continuous flooding, rates of decay were approximately the same as when flooded in the laboratory, where 93 to 100 per cent of the sclerotia were killed after flooding periods of 26 to 31 days. For killing sclerotia this appears to be an efficient method that may be adapted to large areas in south Florida. The necessity for flooding as contrasted to normal weathering was emphasized when sclerotia were placed in commercial fields and subjected only to the prevailing rainfall. Here decay was slow and incomplete, except in one field which was flooded naturally for 6 weeks. This suggests that sclerotia may persist in the soil for considerable lengths of time and that average rainfall in south Florida will not cause appreciable decay except in low lands which may become flooded for several weeks.

SUMMARY

When flooded in marl, muck, or sandy soils, sclerotia of *Sclerotinia sclerotiorum* decayed completely within 23 to 45 days.

Decay was equally rapid during periods of flooding regardless of depth of burial in soil.

Sclerotial decay was as rapid when alternately flooded and drained at intervals of three days as when flooded continuously.

The addition of green organic matter to the soil surface did not hasten rates of sclerotial decay during periods of flooding.

Sclerotia decayed during field flooding at approximately the same rate as under laboratory conditions.

In commercial fields sclerotia decayed slowly and incompletely when subjected only to prevailing rainfall in nonflooded fields.

U. S. DEPARTMENT OF AGRICULTURE
    AND
FLORIDA AGRICULTURAL EXPERIMENT STATION

DAMPING-OFF OF ALFALFA CUTTINGS CAUSED BY RHIZOCTONIA SOLANI[1]

M. F. KERNKAMP, J. W. GIBLER, AND L. J. ELLING

Vegetative propagation is used extensively in the breeding of disease-resistant varieties of alfalfa. In the increase of clonal lines, by stem cuttings, serious losses of cuttings occurred in the greenhouse during the winter of 1947–48. This paper summarizes studies on the organisms concerned, disease development, and control methods.

Various methods of making and rooting alfalfa cuttings have been described by Grandfield, Hansing, and Hackerott (2), Tysdal, Kiesselbach, and Westover (3), and White (4). One of the writers (1) made a rather extensive study of rooting stem cuttings, using various rooting hormones, different types of rooting media, and different types of cuttings. The results of these studies indicated that alfalfa cuttings rooted better in vermiculite[2] than in sand, and that a cutting of single-node and internode length, with all the leaves left on the cutting, was the most desirable type to use. Rooting hormones were of no great value.

During the winter of 1946–47 the cuttings rooted in a highly satisfactory manner; however, during the fall and winter of 1947–48, approximately 50 per cent of the cuttings either failed to root (Fig. 1) or damped off in the greenhouse benches after they had rooted. In some flats the loss was 100 per cent. Grandfield *et al.* (2) reported a similar situation in Kansas and attributed the cause to *Rhizoctonia solani* Kühn and *Ascochyta imperfecta* Peck. The diseased cuttings in our investigations appeared to be similar to those reported by Grandfield *et al.* (2), and *Rhizoctonia solani* was isolated from them.

DISEASE DEVELOPMENT AND SYMPTOMS

Pieces of diseased cuttings were surface-disinfected with 1 per cent sodium hypochlorite and plated on potato-dextrose agar. *Rhizoctonia solani* grew from 28 of 70 platings of internode tissue, while only 1 out of 50 pieces of root tissues yielded *Rhizoctonia*.

Damping-off was first observed in flats of newly established cuttings. The disease progressed from the sides throughout the flats, eventually killing most or all or the cuttings (Fig. 2). This suggested that the causal organism was being harbored in the boards of the flats, and isolations of *Rhizoctonia solani* from the wood of the flats confirmed this.

[2] Vermiculite is a micaceous mineral derived from alteration of mica by heating.

When well-rooted cuttings were transplanted to field soil in greenhouse benches, damping-off developed in patches in the benches.   Visual examination of cuttings showed that the roots were intact, but the portion of the

FIG. 1.   Left, alfalfa cuttings infected with *Rhizoctonia solani*.   Right, healthy cutting.
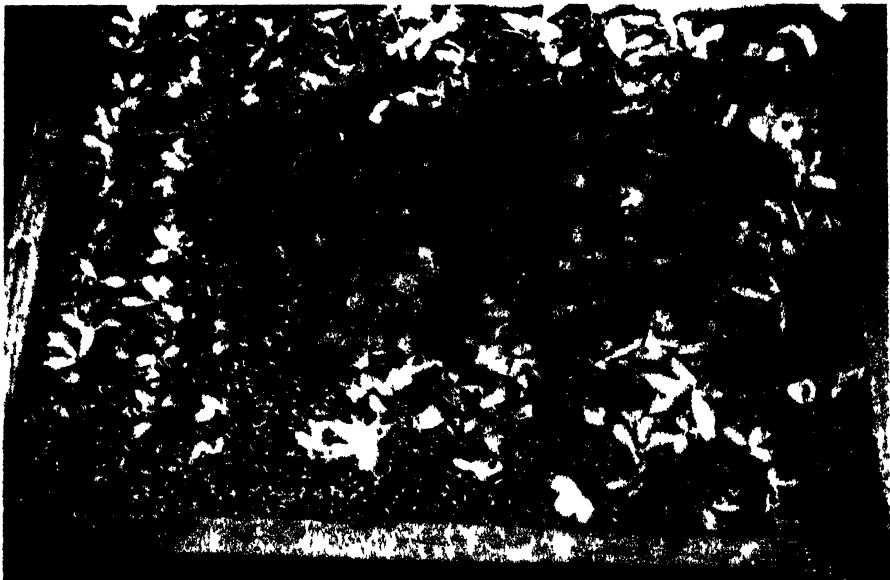
FIG. 2.   Alfalfa cuttings, infected with *Rhizoctonia solani*, in which infection progressed from the sides of the flat leaving a patch of healthy plants in the center.

internodes immediately above the roots was necrotic. The necrosis often extended from the point of root emergence up to the node. In many cases the internode was a brown, soft, hollow cylinder, with the central portion completely rotted away (Fig. 3).
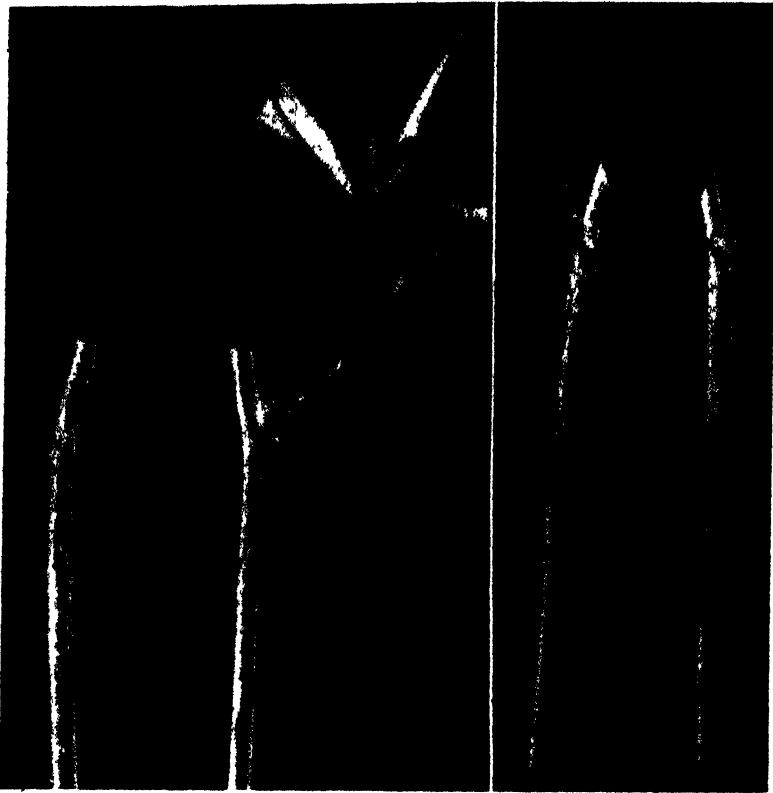
Fig. 3. Left, healthy cutting; pith intact and not discolored. Right, cutting infected with *Rhizoctonia solani*; pith has disintegrated and stem is discolored.

Damping-off was especially severe at high temperatures. For example, many cuttings wilted and died when the temperature rose above 85° F., but when the temperature fell below 75° F. many of the wilted plants recovered temporarily and no further wilting was observed until the temperature again rose above 85° F. This cycle occurred at least three times during periods of fluctuating temperatures in May and June of 1948 in a greenhouse without controlled temperature.

RESULTS OF INOCULATIONS

The observations and isolations made by the writers indicated that *Rhizoctonia solani* was the primary cause of damping-off in these plantings; therefore, inoculations were made with 15 isolates of *Rhizoctonia* that had been obtained from diseased cuttings.

Inoculum was prepared by growing the 15 isolates in a cornmeal-vermiculite medium consisting of 2 teaspoonsful of cornmeal to 1 pint of vermiculite. This medium was sterilized in fruit jars, and the 15 isolates of *Rhizoctonia* were grown in it for 10 to 14 days. Because pathogenically different races of *Rhizoctonia* are known to exist, these isolates were mixed to make a composite inoculum. Since cuttings were to be rooted in vermiculite in flats, ¼ pint of the inoculum was mixed with enough sterilized vermiculite to fill a greenhouse flat with inside measurements of 4 × 13½ × 18¼ in.

In the first test 135 cuttings were planted, comprising 3 replicates of 15 cuttings each of 3 clones of alfalfa. Two days after planting all of the cuttings began to wilt, and 3 days later all were dead. It soon became apparent that the check flats also had become infected, because 10 days after the plantings were made some of the cuttings in the controls began to wilt, and many died a few days later.

Isolations were made from 50 infected cuttings in inoculated flats and 50 infected cuttings in control flats. Every one yielded *Rhizoctonia solani*. Because the organism in the controls apparently came in from the greenhouse bench, the bench and the soil in the bench were partially sterilized with formaldehyde, and the experiment was repeated. Also, in the repetition, the flats were placed on sterile clay saucers, thus preventing them from coming in contact with the soil in the bench. In this test all of the inoculated cuttings became infected and died, while all of the control cuttings rooted and remained healthy.

Wilting in nonautoclaved flats frequently appeared first near the walls of flats (Fig. 2) from which *Rhizoctonia solani* had been isolated from shavings. To test infested flats as a source of inoculum, 12 flats that had been used previously in damping-off tests were washed, and six of them were autoclaved. Autoclaved vermiculite was placed in all 12 flats and 30 cuttings were planted in each flat. Three washed flats and 3 flats that were washed and autoclaved were placed in a greenhouse at 85° F., and a similar series was placed in a greenhouse at 75° F.

In each case the cuttings in the nonautoclaved flats began to wilt within 7 days after planting, whereas the cuttings in the autoclaved flats remained healthy. Thus, infested flats harbor the fungus and are a source of inoculum.

Grandfield *et al.* (2) atomized alfalfa plants with mycelial suspensions of *Rhizoctonia solani* and then planted cuttings from those plants, thus introducing the organism into their rooting medium. The writers duplicated this by covering 90 cuttings with broken pieces of mycelium and then planting them in autoclaved vermiculite. In each case the inoculated cuttings became infected 3 days after planting, while the noninoculated ones remained healthy.

Since rooted cuttings must be transplanted from the rooting medium to*

soil in benches, it is important to know if the causal organism can be transferred with them to the benches. It was not possible to transplant cuttings from inoculated rooting medium (vermiculite) to sterile soil, because the cuttings were always killed if the rooting medium was infested. On other occasions, however, when infected cuttings were inadvertently transplanted to sterile soil in flats, the disease soon spread throughout those flats. This shows that if cuttings become infected in the rooting medium the organism can readily be transferred to greenhouse benches during transplanting and then will spread throughout the bench.

Soil also may be a source of inoculum. This has been shown in many experiments where cuttings rooted in sterile vermiculite were transplanted to inoculated soil or to soil that was naturally infested. In all cases the plantings soon succumbed to *Rhizoctonia*. In one experiment, 100 cuttings were transplanted to inoculated soil. Within 3 days all of the cuttings were severely infected, while the controls remained healthy.

Since temperatures of 85° F. or higher were conducive to disease development, an experiment was made to determine the effect of lower temperatures on development of the disease. Three inoculated flats containing 30 cuttings each were placed in each greenhouse at 60°–65° F., 70°–75° F., and 80°–85° F. A similar series was planted as controls. Damping-off occurred in all of the inoculated flats, the only difference being in rate of disease development. At 60°–65° F. there was very poor rooting and the inoculated cuttings were killed in 23 days; at 70°–75° F. some roots were formed, but the cuttings became infected and died in 16 days; at 80°–85° F. the cuttings failed to root and were killed in 3 days. Cuttings in all of the non-inoculated flats remained healthy.

CONTROL WITH FUNGICIDES

The preceding experiments proved that damping-off of alfalfa cuttings in the greenhouse can be controlled readily by using careful sanitary methods, such as sterilizing flats, rooting medium, greenhouse benches, and soil in benches or flats, and by preventing contact of flats with soil in the benches. These methods may be of no value, however, if *Rhizoctonia solani* is introduced into the rooting medium or soil. Furthermore, since some workers may not have autoclaving equipment available, tests were made to determine whether the application of fungicides to cuttings before planting or to rooting medium and soil after the cuttings were planted would control the disease.

The fungicides were applied by two methods: (1) cuttings were dipped in the dry dusts immediately before planting, and (2) water suspensions of fungicides were sprinkled on the flats after cuttings were planted in vermiculite or after they were transplanted to inoculated soil.

The following fungicides were applied dry to an average of 130 cuttings each: Dithane Z78 (zinc ethylene bisdithiocarbamate), Phygon (dichloro-naphthoquinone), Semesan Jr. (ethyl mercury phosphate), Spergon W

(tetrachlorobenzoquinone), Tersan (tetramethyl thiuram disulfide), Tribasic copper sulfate, and Zerlate (zinc dimethyl dithiocarbamate).

Table 1 is presented in order to give the fungicides, rates of applica-

TABLE 1.—*Fungicides sprinkled on vermiculite or soil, rates of application, and total number of cuttings used in tests*

Fungicide[a]	Grams per square foot	Total number of cuttings in tests
Calo-Clor (mercuric and mercurous chloride)	0.5	180
Dithane Z78 (zinc ethylene bisdithiocarbamate)	1.8	40
Fermate (ferric dimethyl dithiocarbamate)	68.0	40
Phygon (dichloronaphthoquinone)	3.0	360
Phygon-XL (dichloronapthoquinone)	1.3	40
Semesan Jr. (ethyl mercury phosphate)	2.7	90
Spergon (tetrachlorobenzoquinone)	3.0	180
Tersan (tetramethyl thiuram disulfide)	3.0	150
Tribasic copper sulfate (copper basic sulfate)	1.0	150
Zerlate (zinc dimethyl dithiocarbamate)	1.2	40
Leafox 200A (zinc oxide)	11.0	40

[a] None of the fungicides listed gave effective control.

tion, and number of cuttings used in tests in which the fungicides in water suspensions were sprinkled on the flats. Various numbers of cuttings were made from 12 different clones. All of the tests were replicated 3 times and the most promising tests were repeated 2 or 3 times.

None of the fungicides adequately controlled damping-off after *Rhizoctonia solani* had been introduced into the rooting medium or into soil to which rooted cuttings were transplanted. However, Phygon applied dry to cuttings before planting in inoculated flats showed some promise. In these tests, 76 per cent of the cuttings rooted and survived long enough to be transplanted to inoculated soil, but after being in soil several weeks they finally succumbed to *Rhizoctonia*. There was no phytotoxic effect of Phygon applied in this manner.

Since the cuttings were protected to a considerable degree with Phygon, it was considered that this technique might be used safely to partially surface-disinfect cuttings and prevent the introduction of *Rhizoctonia solani* into the rooting medium.

Two hundred seventy cuttings were made and planted in sterile vermiculite. The internodal portions of 90 were covered with mycelium of *Rhizoctonia solani* and planted; 90 were covered with *Rhizoctonia*, dipped into dry Phygon, and planted; and the remaining 90 were planted as a control. Within 7 days all of the inoculated cuttings became diseased, while those that had been inoculated and treated with Phygon, and the controls, all remained healthy and rooted well. In this experiment, as well as in preceding tests, there was no toxic effect of Phygon on the cuttings.

DISCUSSION AND CONCLUSIONS

The results of these experiments demonstrated that *Rhizoctonia solani*

causes damping-off of alfalfa cuttings in the greenhouse, as reported by Grandfield, Hansing, and Hackerott (1). The organism was introduced into greenhouse flats and benches on the cuttings themselves, from infested flats and benches, and from infested soil or vermiculite.

All of the tests in which equipment was autoclaved were successful in controlling the disease. These included autoclaving flats, vermiculite and soil. In addition, partial sterilization of soil in benches with formaldehyde helped to prevent infestation from that source when flats were placed on sterile clay saucers, thus preventing their contact with the soil in the benches.

Fungicides were of no value when sprinkled onto flats and benches after the cuttings were planted. However, Phygon applied dry to cuttings provided some protection to cuttings planted in infested vermiculite, and definitely prevented infection when applied to inoculated cuttings. The latter method is recommended to surface-disinfect cuttings and thus prevent the introduction of the causal organism into the rooting medium.

In 1947–48 approximately 50 per cent of the cuttings were lost because of infection by *Rhizoctonia solani*. In the fall and winter of 1948–49, the writers applied these sanitation methods and obtained very satisfactory control of the disease. The flats were sterilized in an autoclave, new vermiculite was used for the rooting medium, the greenhouse benches were washed with a 5 per cent solution of formaldehyde, fresh field soil was placed in the benches, and the flats were raised above the soil level by placing them on sterile clay saucers. Virtually no loss was sustained in approximately 12,000 cuttings from 70 clones in the rooting medium, as compared to 100 per cent loss in some flats the previous year.

The cuttings were then transplanted to fresh field soil in benches. Five months later no infection could be observed in 55 clones, but the remaining 15 clones had various degrees of infection. These differences were attributed to natural infestation of field soil and different degrees of susceptibility of the different clones, a phenomenon that has been consistently apparent throughout these investigations.

In view of the results of these investigations, the writers recommend that the sanitary methods described herein be applied by those workers who find it necessary to grow alfalfa by vegetative propagation. As a precaution against introducing *Rhizoctonia solani* into the rooting medium, it is further recommended that the internodal portion of the cuttings be dipped in dry Phygon immediately before planting.

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RELATION OF ENVIRONMENTAL FACTORS TO BACTERIAL WILT OF TOMATO[1]

M . E . G a l l e g l y , J r . , a n d J . C . W a l k e r

INTRODUCTION

Bacterial wilt (*Pseudomonas solanacearum* E. F. Sm.) is a disease of tomato, potato, tobacco, and numerous other plants in the southern part of the United States and in various tropical and subtropical countries. It is virtually absent north of the fortieth parallel in North America although Vaughan (4) found that the organism can live over winter in soils at least as far north as central New Jersey. He found that tomato plants became infected at soil temperatures as low as 13° C. but symptoms ordinarily did not become apparent until the temperature was raised to 21°. The rate of disease development increased with increase in soil temperature from 21° to 43°. It was found that disease development was more rapid in soils of high moisture than in soils of low moisture.

The purpose of the present investigation was to compare the effect of various pre-inoculation and post-inoculation environal factors upon development of bacterial wilt of tomato, and to compare the results with similar studies already reported (2, 3) on two other vascular diseases of tomato, i.e., *Fusarium* wilt (*Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyder and Hansen) and bacterial canker (*Corynebacterium michiganense* (E. F. Sm.) H. L. Jensen).

METHODS AND MATERIALS

Seed of the Bonny Best variety of tomato (*Lycopersicon esculentum* Mill.) was planted in enameled pans in clean white silica sand and watered with distilled water. Approximately 3 weeks later, when the first true leaves were forming, the seedlings were transplanted to 8-in. glazed crocks containing a mixture of 3 parts greenhouse compost soil and 1 part sand. Each crock contained 5 plants, and 4 or 5 randomized replicates of one crock each were used for each environal treatment except for cases indicated later where facilities did not permit arrangement of pots in randomized replicates. Predisposing conditions are described for each experiment under Experimental Results.

The culture of the wilt organism was a virulent single-colony isolate kept under sterile mineral oil. Bacteria were transferred from under oil to nutrient-dextrose-agar slants (Bacto-peptone, 10 gm.; dextrose, 10 gm.; beef extract, 3 gm.; agar, 20 gm.; and distilled water, 1,000 ml.). Three

days later the bacterial growth was used to seed several 6-oz. bottle slants. For the inoculum, bacterial growth from twenty 3-day-old bottle-slant cultures was suspended in one liter of distilled water.

After 30 days' growth at the various environal treatments, the plants were removed and their washed roots were dipped in the inoculum, after which they were replanted to their respective crocks. Control plants were treated in the same manner except that distilled water was used in place of the inoculum.

Shortly after the first appearance of symptoms and at varying intervals thereafter the plants were examined and placed in one of the disease classes listed below with its corresponding numerical rating: 0, healthy; 25, slightly wilted; 50, moderately wilted; 75, severely wilted; 100, dead. To secure a disease index, the number of plants in each class was multiplied by its respective rating number, the totals were added, and the sum was divided by the total number of plants in the replicate. The disease indices cited in each experiment are averages of the replicates of a given environal treatment. The results were subjected to analysis of variance.

EXPERIMENTAL RESULTS

Influence of Soil Temperature

Wisconsin soil temperature tanks were used to study the effect of soil temperature. An average air temperature of 22° C. and an optimum soil moisture for host development were maintained. The first experiment extended through November and December and the second through January and February. Tanks were adjusted to maintain soil temperatures of 18°, 24°, 30°, and 36° C. in the first experiment and 24°, 28°, 32°, and 36° C. in the second experiment. At each temperature there were 20 crocks with 5 plants in each crock. Plants were grown at the respective soil temperatures for 30 days and then inoculated. The greatest amount of growth occurred in the range of 28° to 32° C.

The crocks were arranged so that at each post-inoculation soil temperature there were 1 uninoculated and 4 inoculated crocks from each pre-inoculation soil temperature. The indices at the end of the two experiments are presented in tables 1 and 2.

In each experiment the effect of post-inoculation soil temperature was most pronounced. The decided increase in indices was evident in each pre-inoculation group and in the averages from 18° up to 30° in experiment 1 and from 24° to 32° in experiment 2. The rate of disease development was maintained at a nearly constant level above 30° as far as 36°. While some differences appeared between pre-inoculation groups as shown in the figures, they were not consistent in the two experiments and differences between groups were seldom significant. The data show that soil temperature has little or no predispositional effect on the tomato seedlings as it was shown to have in the case of Fusarium wilt (2) and bacterial canker (3).

TABLE 1.—*Effect of pre and post-inoculation soil temperature on development of bacterial wilt of tomato at 30 days after inoculation; experiment 1, November and December*

Post-inoculation temperature	Pre inoculation temperature, °C.				Mean for post-inoculation temperature
	18	24	30	36	
°C	Index	Index	Index	Index	Index
18	17	7	24	28	19
24	34	51	47	65	44
30	71	81	92	96	85
36	88	100	93	97	95
Mean index for pre inoculation temperature	53	55	64	72	

LSD		19 : 1	99 : 1
For comparison of individual indices:		18	24
For comparison of post-inoculation means:		9	12
For comparison of pre inoculation means:		9	12

TABLE 2.—*Effect of pre and post inoculation soil temperature on development of bacterial wilt of tomato at 26 days after inoculation; experiment 2, January and February*

Post-inoculation temperature	Pre inoculation temperature, °C.				Mean for post-inoculation temperature
	24	28	32	36	
°C	Index	Index	Index	Index	Index
24	24	41	43	36	36
28	56	56	40	52	51
32	75	74	69	76	74
36	86	79	73	80	80
Mean index for pre inoculation temperature	60	63	56	61	

LSD		19 : 1	99 : 1
For comparison of individual indices:		15	20
For comparison of post-inoculation means:		7	10
For comparison of pre inoculation means:		NS	NS

Influence of Air Temperature

The effect of air temperature on disease development was studied in a manner similar to that described for soil temperature. Wisconsin temperature tanks were adjusted to give a common soil temperature of 28° C. Twenty-five glazed crocks, 5 plants per crock, were kept at 16°, 20°, 24°, and 28° air temperature for 30 days and then inoculated. The tomatoes grown at 20° and 24° were large and succulent. Those grown at 16° were short and stocky while those grown at 28° were rather spindly. After inoculation, the crocks were rearranged in the manner described for soil temperature studies so that each post-inoculation temperature contained 1 un-

inoculated and 5 inoculated crocks of plants grown at each of the pre-inoculation air temperatures. The first experiment was conducted in January, February, and March, the second in March, April, and May. The final disease indices are presented in tables 3 and 4.

TABLE 3.—*Effect of pre and post-inoculation air temperature on development of bacterial wilt of tomato at 25 days after inoculation; experiment 1, January to March*

Post-inoculation temperature	Pre-inoculation temperature, °C.				Mean for post-inoculation temperature
	16	20	24	28	
°C	Index	Index	Index	Index	Index
16	30	41	44	17	33
20	41	35	47	35	40
24	51	47	45	43	47
28	54	75	69	48	62
Mean index for pre-inoculation temperature	44	50	51	36	

LSD		19:1	99:1
For comparison of individual indices:		17	23
For comparison of post-inoculation means:		9	12
For comparison of pre-inoculation means:		9	12

TABLE 4.—*Effect of pre- and post inoculation air temperature on development of bacterial wilt of tomato at 28 days after inoculation; experiment 2, March to May*

Post-inoculation temperature	Pre inoculation temperature, °C.				Mean for post-inoculation temperature
	16	20	24	28	
°C	Index	Index	Index	Index	Index
16	60	59	43	49	53
20	68	57	43	51	55
24	78	59	73	55	66
28	90	60	65	56	68
Mean index for pre-inoculation temperature	74	59	56	53	

LSD		19:1	99:1
For comparison of individual indices:		19	25
For comparison of post-inoculation means:		9	12
For comparison of pre-inoculation means:		9	12

At a constant favorable soil temperature of 28° C. the supplementary effect of post-inoculation air temperature was not so pronounced as was the effect of soil temperature in the previous experiments. There was a rise in average disease index from 16° to 28° in each experiment. Controlled air temperatures above 28° were not available and it is not to be inferred, therefore, that higher air temperatures are not more favorable for disease development. In the individual pre-inoculation groups the difference in index between temperature increments was often small and insignificant.

The differences between pre-inoculation groups were not consistent in the two experiments. In both experiments the 28° pre-inoculation plants which tended to be spindly usually had the lowest indices. In the winter experiment the 20° and 24° pre-inoculation plants were the most succulent and usually had the highest disease indices. In the spring experiment the 16° pre-inoculation plants were smallest and stockiest and they were consistently the most susceptible, while the 24° pre-inoculation plants which were the largest and most succulent were, contrary to the results in the winter experiment, intermediate in rate of disease development.

It can be concluded from these experiments that the differential effect of air temperature is more effective after inoculation than previous to inoculation in the case of bacterial wilt. It has the same general effect, but in the main is less influential than soil temperature.

Influence of Soil Moisture

To study the effect of soil moisture on disease development, plants were grown at low (40 per cent water-holding capacity), optimum (80 per cent w.h.c.), and high (near 100 per cent w.h.c.) soil moistures. These moisture levels were maintained by adding distilled water to the crocks each morning to bring them up to the desired weights. Plant weights were estimated and additional water was added to correct for the gain in weight. When the plants became larger the crocks were brought to the desired weights twice daily. The first experiment was performed during July, August, and September when the air and soil temperature ranged from 28° to 33° C. Light intensities ranged from 400 to 1600 foot-candles. The day length was adjusted to 18 hr. by use of fluorescent lamps. The second experiment was performed during December, January, and February when day lengths were not controlled. After 30 days' growth the plants were removed and inoculated. Plants grown at the low moisture level were low in vigor and succulence. Plants grown at optimum and high soil moisture levels were equal in fresh weight measurements at the time of inoculation. However, those from the high moisture level were spindly and slightly yellow. At the end of the experiment growth was less at low and at high soil moisture than at the optimum level.

The results are presented in tables 5 and 6. In the first experiment no influence was exerted by pre-inoculation soil moisture. After inoculation, however, the disease index was significantly lower in the plants at low soil moisture than in those in the same pre-inoculation group grown at optimum or high soil moisture. In the second experiment disease development was slightly less in plants that were grown before inoculation at the low moisture level. After inoculation the disease indices were lower in the plants at low and optimum soil moisture than in those in the same pre-inoculation group grown at high soil moisture. In both experiments, disease development in plants grown at high soil moisture after inocula-

tion was greater than in those grown in low soil moisture. This is in accord with Vaughan (4), who found that bacterial wilt developed more rapidly in high soil moisture than in low soil moisture.

TABLE 5.—*Effect of pre- and post-inoculation soil moisture on development of bacterial wilt of tomato at 34 days after inoculation; experiment 1, July to September*

Post-inoculation soil moisture	Pre-inoculation soil moisture			Mean for post-inoculation soil moisture
	Low	Optimum	High	
	Index	Index	Index	Index
Low	53	58	55	55
Optimum	81	84	85	83
High	71	84	85	80
Mean index for pre inoculation soil moisture	68	75	75	
LSD			19:1	99:1
For comparison of individual indices:			20	27
For comparison of post-inoculation means:			12	16
For comparison of pre-inoculation means:			NS	NS

TABLE 6.—*Effect of pre- and post-inoculation soil moisture on development of bacterial wilt of tomato at 27 days after inoculation; experiment 2, December to February*

Post-inoculation soil moisture	Pre-inoculation soil moisture			Mean for post-inoculation soil moisture
	Low	Optimum	High	
	Index	Index	Index	Index
Low	24	51	56	44
Optimum	49	47	41	46
High	55	59	76	63
Mean index for pre-inoculation soil moisture	43	52	58	
LSD			19:1	99:1
For comparison of individual indices:			22	NS
For comparison of post inoculation means:			14	NS
For comparison of pre inoculation means:			NS	NS

Influence of Day Length

To study the effect of photoperiod in predisposing tomato plants to bacterial wilt, cages covered with black 14-lb. building paper were used to adjust day length. One group of 15 crocks of plants was covered with a black cage from 4 P.M. until 10 A.M., a second group was covered from 7 P.M. until 7 A.M., and a third was left uncovered. Fluorescent lamps were regulated to burn from 4 A.M. until 10 P.M. Thus plants were grown at day lengths of 6 hr., 12 hr., and 18 hr. Soil moisture was kept near the optimum level. After 30 days' growth the plants were removed and inoculated. Plants grown at the 6-hr. day length were small, yellow, and spindly while those grown at 12 hr. appeared healthy but were shorter and less suc-

TABLE 7.—*Effect of pre- and post-inoculation day length on development of bacterial wilt of tomato at 33 days after inoculation; experiment 1, July and August*

Post-inoculation day length	Pre-inoculation day length			Mean for post-inoculation day length
	6-hr.	12-hr.	18-hr.	
Hours	Index	Index	Index	Index
6	81	88	91	87
12	75	66	74	72
18	63	49	65	59
Mean index for pre-inoculation day length	73	68	77	
LSD			19:1	99:1
For comparison of individual indices:			35	NS
For comparison of post-inoculation means:			20	NS
For comparison of pre-inoculation means:			NS	NS

culent than those grown at the 18-hr. day length. After inoculation, the crocks were rearranged so that each post-inoculation length of day contained 1 uninoculated and 4 inoculated crocks of plants which were predisposed at 6-hr., 12-hr., and 18-hr. day lengths. The first experiment was performed during July and August when air and soil temperatures ranged from 28° to 32° C. The second experiment was carried out from September to October, when average light intensities were lower than in the first experiment.

In the midsummer experiment (Table 7) there were no appreciable differences between pre-inoculation day-length groups. There was, however, a consistent downward trend in index with increase in post-inoculation day length for each pre-inoculation group. In the autumn experiment (Table 8) when light intensities were lower, there was a trend toward reduction in index with increase in pre-inoculation day lengths, but only when the post-inoculation day lengths were 12 or 18 hr. As in the mid-

TABLE 8.—*Effect of pre- and post-inoculation day length on development of bacterial wilt of tomato at 26 days after inoculation; experiment 2, September and October*

Post-inoculation day length	Pre-inoculation day length			Mean for post-inoculation day length
	6 hr.	12-hr.	18-hr.	
Hours	Index	Index	Index	Index
6	76	71	90	79
12	50	33	20	34
18	55	14	8	26
Mean index for pre-inoculation day length	60	39	39	
LSD			19:1	99:1
For comparison of individual indices:			23	32
For comparison of post-inoculation means:			14	18
For comparison of pre-inoculation means:			14	18

summer experiment there was a definite trend of reduction in disease index with increase in post-inoculation day length. It appeared that there was some interaction between light intensity and day length in predisposition of tomato plants to bacterial wilt.

Influence of Light Intensity

In some of the previous experiments there were distinct indications that light intensity influenced the interaction of host and parasite. In the air temperature series there was little difference between pre-inoculation groups in the midwinter experiment, while in the spring experiment when light intensity was greater the groups predisposed at 20°, 24°, and 28° C., were significantly lower in disease index than those predisposed at 16°. In the length-of-day experiments the differences between post-inoculation groups and between pre-inoculation groups were greater in the autumn experiment than in the midsummer experiment. In the soil moisture experiments the differences between pre-inoculation groups were greater in the winter experiment than in the midsummer experiment.

Twelve crocks of plants were grown under cheesecloth and 12 were grown near-by on the greenhouse bench to give low and normal light intensities, respectively. The light intensity at normal ranged from 400 foot-candles on cloudy days to 1600 foot-candles on bright days, while at low light the intensities ranged from 100 to 400 foot-candles. Light intensity readings were made with a General Electric Exposure Meter equipped with multiplying masks. Optimum soil moisture conditions were maintained. After 26 days' growth the plants were removed, weighed, and then inoculated. The plants grown under low light were small and spindly while those grown under normal light were large and very succulent. After inoculation, 1 crock of uninoculated and 5 of inoculated plants from each pre-inoculation light intensity were placed under both low and normal light conditions. The first experiment was performed during July and August with fluorescent lights supplementing daylight to give an 18-hr. day length The second experiment was in October and November. A third experiment was run in December and January.

In table 9 are shown the effects of reduced and normal post-inoculation

TABLE 9.—*The effect of post inoculation light intensities on susceptibility of plants grown at normal light intensity before inoculation*

Time of year	Post-inoculation light intensity		LSD 19:1
	Reduced	Normal	
	Index	*Index*	
July–August	61	39	19
October–November	45	24	14
December–January	92	64	

light intensities on the development of disease in plants grown previous to
inoculation at the current normal light intensity; the results are from mid-
summer, autumn, and midwinter experiments. It may be seen that in each
environment reduced light intensity increased severity of disease develop-
ment. In table 10 the effect of pre-inoculation light intensity is shown when

TABLE 10—*The effect of pre-inoculation light intensities on susceptibility of plants
grown at normal light intensity after inoculation*

Time of year	Pre-inoculation light intensity		LSD 19:1
	Reduced	Normal	
	Index	*Index*	
July–August	26	39	NS
October–November	49	24	14
December–January	65	64	

applied at the same three seasons. In midsummer when normal light in-
tensity was highest and in midwinter when it was lowest, pre-inoculation
light intensity had little effect. In autumn pre-inoculation reduction in
light intensity had the same effect as post-inoculation light intensity.

DISCUSSION

It is clear that environal conditions after inoculation were of primary
importance in determining the rate of development of bacterial wilt of to-
mato. Experiments with soil temperature showed that in each pre-inocu-
lation group there was an increase in rate of disease development with an
increase in post-inoculation temperature. Post-inoculation air temperature
had the same general effect as post-inoculation soil temperature, but in the
main it was less influential than soil temperature. In experiments with
soil moisture the data showed that disease development was higher in plants
grown after inoculation at high soil moisture than in those grown at low
soil moisture. In each pre-inoculation day-length group there was a con-
sistent increase in disease development with decrease in post-inoculation
day length. Post-inoculation results with low and normal light intensity
showed an increase in disease development in the former light.

The effects of pre-inoculation environal factors on development of bac-
terial wilt were not so pronounced and consistent as the effects of post-in-
oculation environment. The first experiment with soil temperature showed
that tomatoes predisposed at high soil temperature were more susceptible
than plants predisposed at low temperature. However, results of the sec-
ond experiment showed pre-inoculation temperatures to have little or no
effect on disease development and thus failed to confirm those of the first
experiment. In both of the experiments where the predisposing air tem-
perature was varied, disease development was influenced but it was obvious
that the uncontrolled factors at the two periods in the year modified the re-

sults considerably. In the winter experiment the 20° and 24° C. pre-inoculation plants were the most succulent and usually had the highest disease indices. In the spring experiment the plants predisposed at 16° air temperature were the smallest and stockiest and they were consistently the most susceptible. Contrary to the results of the first air temperature experiment the 24° pre-inoculation plants which were the largest and most succulent were intermediate in rate of disease development. In both experiments the plants predisposed at 28° air temperature were consistently less susceptible than those predisposed at each of the lower temperatures.

Various levels of soil moisture had no predisposing effect on disease development in an experiment performed during the summer; in a winter experiment plants predisposed at low soil moisture were slightly less susceptible but not significantly so. In the first length-of-day experiment, performed during the summer, pre-inoculation day lengths failed to alter disease development; but in the second experiment, performed during late fall, plants predisposed at day lengths of 6 hr. were more susceptible than those predisposed at day lengths of 12 and 18 hr. In midsummer when light intensity was highest and in midwinter when light intensity was lowest, pre-inoculation light intensity had little effect on disease development. In autumn when light intensity was intermediate, plants predisposed at low light intensity were more susceptible than those predisposed at normal light.

It is of interest to compare the post-inoculation results with those secured by essentially the same methods with Fusarium wilt and bacterial canker of the same host (2, 3). Development of Fusarium wilt and bacterial canker increased with an increase in soil temperature up to 28° but they were decreased when temperatures were increased to 36° and 34° C., respectively; bacterial wilt increased with an increase in soil temperature up to 36°. Thus the optimum temperature for development of bacterial wilt was higher than it was for the other two diseases. Bacterial wilt increased with an increase in post-inoculation air temperature from 16° to 28° C., while with bacterial canker there was little difference due to the same temperatures when the soil was maintained at a constant temperature. The results with Fusarium wilt cannot be compared with the other two since the soil temperature was allowed to vary with the air temperature. With bacterial canker it was found that disease development after inoculation was greater at low and optimum soil moisture; with Fusarium wilt, Clayton (1) found that disease development was greater at optimum soil moisture. Bacterial wilt development was higher at optimum and high soil moistures. The point of particular import here is that post-inoculation effects of soil moisture on bacterial wilt and on bacterial canker were diametrically opposite. Low light intensity after inoculation was more favorable to development of bacterial wilt and less favorable to development of bacterial canker than normal light intensity. Thus, light intensity also was diametrically opposite in its effect on these two diseases. Post-inoculation day-length studies were not conducted with Fusarium wilt and bacterial canker.

SUMMARY

Young tomato plants of the variety Bonny Best were subjected to various environments for a 30-day period before inoculation with the bacterial wilt organism (*Pseudomonas solanacearum* E. F. Sm.). In each experiment only one factor of environment was varied. After inoculation the plants were rearranged and again subjected to various degrees of this factor. Thus results were obtained which showed the effect of post-inoculation and pre-inoculation environal factors on disease development.

Post-inoculation results showed that bacterial wilt increased with an increase in soil temperature and with an increase in air temperature up to 36° and 28° C., respectively. Air temperatures above 28° were not studied. Disease development was greater after inoculation at low light intensity and short day lengths than at respective normal light intensity and longer day lengths. At optimum and high soil moisture levels, disease development was greater than at low soil moisture.

Pre-inoculation environal factors were not so influential in affecting disease development as were post-inoculation factors. In one experiment with soil temperature there was an increase in disease with an increase in pre-inoculation temperature. In a second experiment, predisposing soil temperatures had little effect on disease development. Plants that were predisposed at 28° air temperature were less susceptible than those predisposed at lower temperatures. In one experiment with varied light intensities, plants predisposed at low light were most susceptible. In two other experiments, pre-inoculation light intensity had little effect. Results of one experiment with varied lengths of day showed a decrease in disease with an increase in predisposing day lengths. Results of another experiment showed predisposition to have little effect on disease development. Predisposing soil moisture levels had little effect on wilt development.

Results of these experiments indicate that environal conditions after inoculation are of primary importance in determining the development of bacterial wilt in tomato plants.

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PHYTOPATHOLOGICAL NOTES

Ascochyta pinodella Footrot of Peas in Western Washington.[1]—The intensification of pea growing for canning and freezing in western Washington has been accompanied by an increase in prevalence and destructiveness of footrot organisms. Studies made from 1943 to 1946 showed that *Ascochyta pinodella* Jones was responsible for approximately three-fourths of the loss, with *Aphanomyces* sp., *Fusarium* spp., and *Rhizoctonia* sp. accounting for most of the remainder. Pea wilt Fusaria were encountered only in limited areas.

The destructiveness of footrot varied from year to year even within the same fields in conjunction with climatic variations. During cool and moist summers almost normal yields were obtained in certain fields in which practically every plant was attacked. Yet during hot dry summers the yield from such fields was severely reduced.

Analyses[2] of soil samples taken from fields infested to different extents did not show any correlation between the severity of the disease and pH, nitrogen, potash, calcium, or organic content. In 1943, there was a slight correlation between low phosphorus and a small amount of footrot, as compared to high phosphorus and considerable footrot. However, the correlations were not striking.

While studying the pathogenicity of single-spore cultures of the various isolates, it was noted that considerable sectoring and variation in cultural appearance occurred in *Ascochyta pinodella*. Such variation was expressed by rapid *vs.* slow growth, dark *vs.* light mycelium, appressed *vs.* matted mycelium, *etc.* Some of the variation and sectoring can be observed in figure 1. A variation in pathogenicity between different isolates was also encountered, but no attempt was made to correlate this with cultural characteristics.

Tests of resistance were made in the hope of obtaining a variety suitable either for growing or for breeding purposes. Over 500 named and unnamed varieties or strains[3] were tested in the greenhouse and/or field by pouring on the seeds at planting time suspensions of the macerated fungi cultured on agar or barley. Most of the tests were performed in duplicate or quadruplicate and repeated at least once with all varieties and more often with the most promising ones. The plants were dug after

blooming and graded into three or four lots based upon the degree of severity of footrot. From this an index of infection was obtained.

Many of the difficulties listed by Weimer[4] were encountered in the testing program and similar allowances have had to be made in interpreting the data. None of the varieties given adequate tests could be considered immune. However, certain ones possessed considerable resistance to various isolates of *A. pinodella* under western Washington conditions. Among these was Bangalia (Crites Moscow)[5] which Weimer[4] listed as very susceptible under Georgia conditions to a combination of *A. pinodella* and

FIG. 1. Sectoring of single-spore isolates of *Ascochyta pinodella* grown on potato-dextrose agar.

Mycosphaerella pinodes (B. and B.) Vest. However, the Lawyers[6] have stated that varieties may react differently to these two fungi even though the latter are closely related.

Dwarf Gray Sugar (Northrup King) was the most promising of all varieties tested adequately. One of the Shoemaker strains from Honan, China (labelled No. 914; Acc. No. 27625-2), was very promising in a series of tests with a few seeds. Likewise VA2, a selection (by the Lawyers) from Weimer's II 251-11-E (Austrian Winter × FC 18074), was promising in all but one of the tests on a few seeds. The Lawyers[6] and Weimer

[4] J. L. Weimer. Resistance of *Lathyrus* spp. and *Pisum* spp. to *Ascochyta pinodella* and *Mycosphaerella pinodes*. Jour. Ag. Res. [U.S.] 75: 181–190. 1947.

[5] Company furnishing the seed.

[6] Lewis and Adele Lawyer. Personal communication.

have reported H 251-11-E to be slightly resistant to *M. pinodes.* Others of the Shoemaker collection having a certain degree of resistance were: D'Hollandia (655), DeGrace White Dwarf (656), Peulen de Grace (861), and Chang (894). The two market types with some resistance were Horsford Market Garden (Associated Seed Growers) and Famous (Rogers Bros.).

Rotation appears to be the only practicable control measure at the present time for *Ascochyta pinodella* footrot of peas in western Washington. Changing the soil fertility would probably not influence development of the disease appreciably, in view of results of soil analyses from infected and healthy fields. Soil sterilization would probably be effective but prohibitive in cost. Therefore, the development of a resistant variety seems to offer the most hope for a long-time program, and this may be complicated by the apparent variation in the organism.

In this study not a single variety or strain of peas could be found that might be considered immune from attack by *A. pinodella,* with the possible exception of No. 914 in which only a few seeds were available for testing. However, a few types were found that appeared to be partially resistant. The best of these are starchy or field types and therefore can be expected to give some trouble in breeding for a suitable sugar type. Meanwhile it is suggested that pea growers in western Washington with severely infested land experiment with the varieties Horsford Market Garden and Famous which had a fair amount of resistance in our tests.— CHARLES J. GOULD, Western Washington Experiment Station, Puyallup, Washington.

New Hosts for Botrytis cinerea and B. elliptica in the Pacific Northwest.[1]—During the past year *Botrytis cinerea* Pers. ex Auct. and *B. elliptica* (Berk.) Cke. were observed on new hosts. *B. cinerea,* the common gray mold, was found on *Gerbera Jamesonii* Hooker. *B. elliptica* was observed causing a leaf and petal spotting on *Erythronium grandiflorum* Pursh var. *pallidum* St. John.

On *Gerbera Jamesonii* (WSC-PP No. 19897)[2] growing in one of the local greenhouses, *Botrytis cinerea* was causing a very severe crown rot. The soil in which these *Gerbera* plants were growing had been used previously to grow a crop of *Ranunculus* sp. Although the *Ranunculus* had been severely infected with *B. cinerea,* the soil was not sterilized before being used for the *Gerbera.* Initial infection occurred at the crown. From this point the fungus spread both laterally and downward. Infected petioles showed long, slightly sunken, dark lesions. The leaves wilted, soon turned pale yellow, and died. The fungus was found sporulating heavily on all infected parts. Severely infected plants were completely killed.

Both microscopic mounts and isolations showed the typical cinereous, sub-globose spores of *B. cinerea*. The conidia measured 6.5–12 × 5.0–7.5 μ (mean 11.5 × 7.0 μ).

Botrytis elliptica, the well-known cause of a foliage spot of liliaceous plants, was found associated with a stem, leaf, and petal spotting of *Erythronium grandiflorum* var. *pallidum* (WSC-PP No. 19898)[2] growing in its native habitat (open stand of *Pinus ponderosa* Dougl., 1 mi. southeast of Peola, Garfield Co., Wash.). The spots were small (⅓ to 2 mm. diameter) and circular, and possessed dark brown, slightly raised borders. The centers of the spots were light tan. On the more severely infected portions of the plants, the spots had coalesced to form large, dark brown, necrotic areas. On the stems, the symptoms were expressed as elongated spots which coalesced to form streaks (Fig. 1). Grayish spore masses were found on many affected parts. The elliptical to elongate, hyaline conidia measured 12–22 × 10–14 μ (mean 17.7 × 11.7 μ).—NEIL ALLAN MacLEAN and CHARLES GARDNER SHAW, Department of Plant Pathology, State College of Washington, Pullman, Washington.

Rhizoctonia Canker of Tomato.—The winter growing season of 1948–1949 in Dade County, Florida, was notable for the widespread, and in many cases severe, attacks of *Rhizoctonia solani* Kühn on vegetable crops. Losses of green-wrap tomatoes due to soil rot were unusually high, amounting to as much as 50 per cent of certain pickings of experimental plantings. Of particular interest on tomato, however, was the formation of cankers at the base of branches by this parasite. Since no reference to this has been found in the available literature, a description is presented herewith.

Under natural conditions this canker apparently results from the growth of *Rhizoctonia solani* into the stem through a leaf in contact with the soil. The outline of the canker varies from nearly circular to a partial or complete girdling band around the branch about the width of the leaf base (Fig. 1, A–C). Frequently the lesion is marked with alternating bands of dark-brown to red-brown and light-brown or tan. The margin is sharply delimited and the affected area is sunken. Partially or completely girdled branches sometimes wilt and are especially subject to wind or mechanical damage. In a vigorous plant, penetration of the fungus is apparently limited to the cortex and outer vascular cylinder. With decline of the plants the canker rapidly enlarges longitudinally along the stem and branch (Fig. 1, D). Penetration of the vascular cylinder and pith occurs with lesion elongation, and girdled branches are killed.

Isolations from the margins and from the pith of elongated cankers resulted uniformly in pure cultures of *Rhizoctonia solani*. While no sclerotia were found on the lesions, they were formed abundantly on potato-dextrose agar. In choosing plants to be inoculated, care was taken to select branches subtended by healthy leaves not in contact with the soil. The

inoculum (sclerotia or agar disks containing actively growing hyphae) was inserted into slits made in the petioles about 2 in. from the stem. The inoculated area was then covered with cellophane tape. Control plants were treated similarly except that no inoculum was introduced. Ten days later the inoculated leaves were dead and cankers, indistinguishable from those

Fig. 1. Rhizoctonia canker of tomato. A. Canker developing around petiole base. B, C. Complete and partial girdling of branch by *R. solani*. D. Enlarged canker seen during plant decline.

occurring naturally, were developing on the stem. The control plants were unaffected by the treatment. Isolations from the cankers produced as a result of inoculation yielded cultures of *R. solani* indistinguishable from subcultures of the original inoculum.—ROBERT A. CONOVER, University of Florida Sub-Tropical Experiment Station, Homestead, Florida.

Note:—Remove and insert in your copy of Vol. 38, No. 12, December 1948 issue.

ADDITION TO THE INDEX FOR VOLUME 38, PUBLISHED IN THE DECEMBER 1948 ISSUE

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THE EFFECT OF THE SUGAR CONCENTRATION ON CONIDIAL SIZE OF SOME SPECIES OF HELMINTHOSPORIUM[1, 2]

Edward S. Elliott

As part of a more extensive study of factors influencing the sporulation of fungi, the effect of the sugar concentration in the medium on the size of conidia of some species of *Helminthosporium* has been investigated.

Taxonomic descriptions of spore size as given by different workers are often found to vary markedly. Pammel, King, and Bakke (5) have described *Helminthosporium sativum* as having conidia, in nature, which measure 105 to 130 μ in length and contain 7 to 14 cells. Dosdall (2) described the conidia of this species as being 56 to 83 μ long, while Stakman (7) found the conidia of three isolates to vary in average length from 35 to 60 μ.

Undoubtedly, some of the reported variation is due to biologic forms of this species, as has been shown by Christensen (1). He found at least four biologic forms of *Helminthosporium sativum* which differed in their degree of pathogenicity, in physiological characteristics in culture, and in the size and number of septations of conidia.

Lack of uniform methods of culturing has probably contributed more to these differences than any other factor. Sherbakoff (6) found the conidial size of several species of *Fusarium* to vary widely when cultured on different media.

This paper reports the effect of the quantity of sugar in the medium on the size of conidia produced by some species of *Helminthosporium*. It is believed that these investigations will explain, in part, why such variation in spore size has been reported by different investigators. The need for more standardized culture methods is indicated.

MATERIALS AND METHODS

The cultures of *Helminthosporium* used in this work included *H. victoriae* M. and M., isolated from Vicland oats grown in northern West Virginia, *H. sativum* Pam., King, and Bakke, *H. turcicum* Pass., *H. oryzae* Breda de Haan, and *H. sigmoideum* var. *irregulare* Cralley and Tullis. All produced conidia readily on several media.

Three kinds of media were used: malt extract, a glucose-casein hydrolysate medium as described by Leonian and Lilly (4), and a glucose-nitrate

953

medium consisting of sodium nitrate ($NaNO_3$), 2.0 gm.; potassium phosphate (K_2HPO_4), 1.0 gm.; potassium chloride (KCl), 0.5 gm.; magnesium sulfate ($MgSO_4 \cdot 7H_2O$), 0.5 gm.; ferrous sulfate ($FeSO_4 \cdot 7H_2O$), 0.1 gm.; double distilled water to make 1000 ml.; glucose, variable. All media contained 20 gm. agar per liter.

The sugar content of the media was varied but the nitrogen source and other components remained constant, except for the malt extract medium. The necessary amount of malt extract was added to double distilled water and the entire medium was diluted to secure the various sugar concentrations. The desired glucose content of the other media was secured by preparing the sugar solution separately and adding the proper amount to each Petri dish. Water was then added to make the total volume 24 ml. The media were sterilized for 15 min. at 15 lb. pressure. Small pieces of mycelium and spores used for inoculum were taken from cultures growing on agar slants in test tubes.

The cultures were incubated at $25° \pm 1°$ C. in diffuse light. They were allowed to reach maturity before the conidia were measured; this generally required 20 to 30 days. Conidia were produced sooner on media containing low concentrations of sugar. A camera lucida and a calibrated scale were used to measure the length of conidia.

The length of conidia was correlated with the number of cells in the conidia. Both measurements (length and number of cells) were used in most cases to determine the average size of conidia, one being used as a check on the other. Width of conidia also varied, but no attempt was made to correlate this with the length and number of cells.

Since the object of this work was to determine the variation in the size of conidia produced on media containing different quantities of sugar, only 25 conidia were measured. The average length of numerous groups of 25 conidia taken at random from the same culture varied but little. Where there was doubt that the average length of 25 was not in close agreement, 50, 75, or 100 conidia were measured. All the conidia in each microscopic field under the high power objective were measured unless the number exceeded the desired total of 25. If less than the desired number were present in the field, other fields selected at random were used.

Since the differences obtained in spore size were so great, it was not considered necessary to treat the data statistically.

RESULTS

Helminthosporium victoriae. Preliminary experiments with malt extract medium showed that the length and number of septations of the conidia were inversely proportional to the concentration of malt extract. Measurement of 100 conidia from each medium substantiated these preliminary observations. The 2 per cent malt extract agar yielded conidia which meas-

ured 22.2 (10–60) μ long,[3] with 2.8 (1–7) cells[3]. Conidia from 0.5 per cent malt extract agar medium measured 35.7 (15–50) μ and contained 5.1 (2–8) cells. Those from 0.1 per cent malt extract agar medium measured 59.1 (43–78) μ and contained 7.4 (5–9) cells.

The results of the preliminary studies were confirmed by measuring conidia of *Helminthosporium victoriae* produced on three different solid agar media—malt extract, casein hydrolysate, and glucose nitrate—each
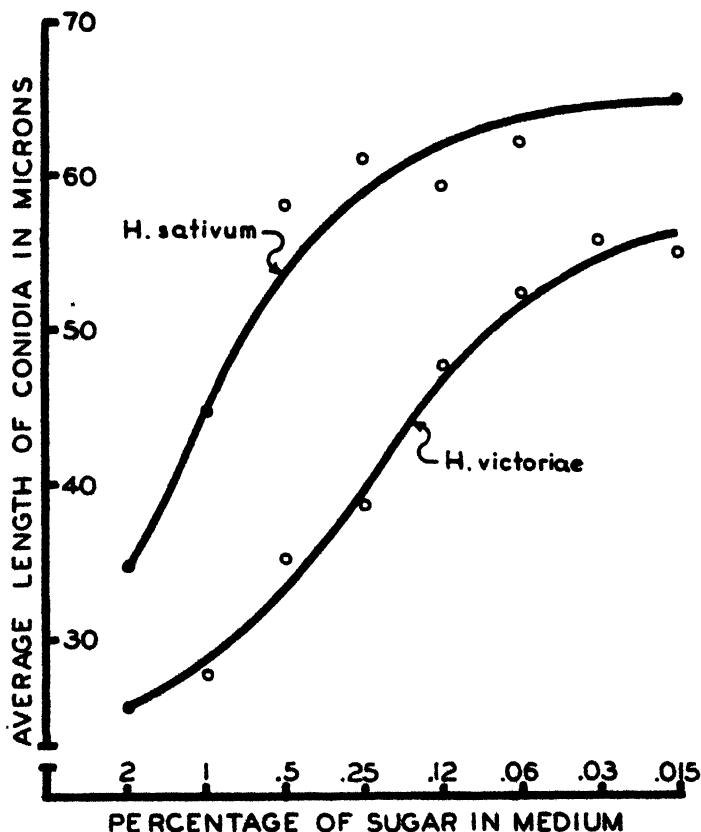
FIG. 1.   The trend in average length of *Helminthosporium sativum* and *H. victoriae* conidia from cultures on glucose-nitrate medium containing different sugar concentrations.

having 8 different sugar concentrations ranging from 2 per cent to 0.015 per cent.   The average length of conidia varied from a minimum of 25.8 μ on 2 per cent sugar to a maximum of 56.0 μ on 0.03 per cent sugar.

Each of the three media yielded conidia which averaged about the same length providing there was an equal amount of sugar in each.   The close correlation between the average length of conidia and the sugar concentration of the glucose-nitrate medium is illustrated in figure 1.   A similar correlation between the average number of cells and the sugar concentration for each medium is shown in table 1.

[3] Average, minimum, and maximum measurements and cell count indicated.

Helminthosporium sativum. Malt extract and glucose-nitrate agar media, having 8 sugar concentrations ranging from 2 per cent to 0.015 per cent, were used for cultures of *H. sativum*. When the average length of conidia was determined for each concentration it was found that the trend in average length was similar to that described for conidia of *H. victoriae*. As in *H. victoriae* there was a correlation between the length of conidia and the number of cells that they contained. The average length of conidia produced on different amounts of sugar in the basal glucose-nitrate medium is shown in figure 1. The average number of cells in the conidia were similar (Table 1) for the same dilution of each medium.

TABLE 1.—*The effect of various amounts of sugar in the media on average number of cells contained in conidia of Helminthosporium victoriae and H. sativum*

| Percentage of sugar in medium | Average number of cells[a] in conidia | | | |
| | H. victoriae | | H. sativum | |
	Glucose nitrate	Malt extract	Glucose nitrate	Malt extract
2.0	2.8 (1– 4)	3.4 (1– 6)	4.1 (1– 9)	4.2 (2– 7)
1.0	3.8 (2– 8)	3.8 (2– 6)	5.6 (4– 9) .	5.0 (2– 9)
0.5	4.9 (4– 9)	4.5 (2– 7)	6.7 (5– 9)	6.2 (2– 9)
0.25	5.2 (3– 7)	5.6 (3– 8)	7.1 (5– 9)	6.8 (3–10)
0.12	6.3 (4– 8)	7.1 (5–10)	7.0 (4– 9)	7.6 (4–11)
0.06	7.1 (5– 9)	7.2 (5– 9)	7.4 (6–10)	7.7 (6– 9)
0.03	7.3 (6– 9)	7.8 (7–10)		7.7 (6– 9)
0.015	7.3 (4–10)	7.7 (5–10)	7.6 (6–11)	7.3 (5–10)

[a] Figures in parentheses give minimum and maximum number of cells in conidia.

Since the media containing 2 per cent sugar produced conidia of the minimum average size, another series of glucose-nitrate media containing larger amounts of sugar was used to find if more sugar would further reduce the average size. Media containing 2 per cent, 8 per cent, and 16 per cent sugar produced conidia which averaged 35.0 μ, 32.8 μ, and 33.0 μ, respectively. A further increase in sugar above 2 per cent did not change the minimum average length to any extent but tended to reduce the total number of conidia.

Other species. Cultures of *Helminthosporium sigmoideum* var. *irregulare* grown on glucose-nitrate medium of 8 different sugar concentrations varying from 2 per cent to 0.015 per cent produced conidia which followed the trend described for *H. victoriae* and *H. sativum*. Since conidia of this species are small and relatively uniform in length, the variation in average size from high to low sugar concentrations was not so great as in the previously studied species. Although there was some indication that conidia of *H. turcicum* varied in average length depending upon the amount of sugar in the medium, the results were not conclusive. A marked decrease in average size of conidia of *H. oryzae* was found only on media containing more than 4 per cent sugar. The average size was reduced from 93 μ on 4 per cent sugar to 59 μ on 16 per cent sugar.

DISCUSSION

In these experiments, conidial length of four species of *Helmintho-sporium* was found to be regulated by the quantity of sugar in the medium. The average length of conidia was inversely proportional to the amount of sugar available. The range in sugar concentration over which the variation in average length occurs is not the same for each species. An increase in the amount of sugar beyond that required to produce conidia of the minimum average length, or a decrease beyond that required to produce the maximum average length, serves only to reduce the total number of conidia. Under the cultural conditions provided, time of incubation had no apparent effect on the size of conidia produced. Mycelial growth increased with increasing quantities of sugar, which may partially explain the reduction in size of conidia if we assume that food materials are utilized for vegetative growth rather than sporulation when an abundant supply is available. Whether the size and number of conidia produced in nature on the host plant is related to the sugar content at various stages of growth of the host is a problem which would require further study.

The number of cells contained in the conidia was directly proportional to the length of conidia. A study of the number of cells alone would give results which would closely approximate those obtained by measuring spore length.

Horne and Mitter (3) in studying some species of *Fusarium* arrived at similar conclusions regarding the effect of glucose on the sporulating capacity and the frequency of septation. They have reported that by increasing the glucose concentration either the sporulating capacity was reduced or the average septation number was lowered, or both. With one strain of *F. culmorum*, spore production gradually fell off as the glucose increased from an initial concentration of 2 per cent to a concentration of 20 per cent. The average septations in this case remained unaltered. With another strain of *F. culmorum* both spore production and the average septation declined within this range. The number of septations in spores of *F. poly-morphum* declined sharply as glucose concentration increased from 2 per cent to 8 per cent.

The obvious differences in the size of conidia formed on media having different sugar contents indicates one reason, at least, why taxonomic descriptions of spore size are often not in agreement. This variation probably is not peculiar to the genus *Helminthosporium*, but may also occur in other fungi.

These data indicate that whenever size of conidia formed in culture is reported, the exact composition of the medium and other cultural conditions should be given. This seems particularly true for groups in which the species or varieties may be separated on the basis of conidial measurements.

SUMMARY

The conidial size of four species of *Helminthosporium* in culture was influenced by the quantity of sugar in the medium. Within limits, the length of conidia was inversely proportional to the amount of sugar available to the fungus. The numbers of cells in the conidia were generally found to be directly proportional to the length of conidia.

H. victoriae produced conidia of maximum average length on media containing 0.03 per cent sugar, and conidia of minimum average length on media containing 2 per cent sugar. The average length on 0.03 per cent sugar was more than double the average on 2 per cent sugar.

H. sativum produced conidia of maximum average length on media containing 0.12 per cent sugar. Conidia that were approximately half that size, the minimum average length, were produced on media containing 2 per cent sugar.

The average size of conidia of *H. oryzae* was markedly decreased only on media containing more than 4 per cent sugar.

Since conidia of *H. sigmoideum* var. *irregulare* are small and relatively uniform in length, the variation in average length from high to low sugar concentrations was not great, but it followed the trend already described.

No conclusive results were obtained to show that conidia of *H. turcicum* vary in average size on media of different sugar concentrations.

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INHERITANCE OF RESISTANCE TO LOOSE SMUT, USTILAGO NUDA, IN BARLEY[1]

C. W. SCHALLER[2]

INTRODUCTION

Loose smut of barley, caused by *Ustilago nuda* (Jens.) Rostr., is an important disease in many barley-producing areas, particularly in the humid and subhumid areas of production. While not so prevalent as some diseases, the lack of easily applied control measures increases its importance in areas where it does occur. The reduction in yield is approximately in direct proportion to the percentage of smutted plants (13). Although loose smut can be controlled by treating the seed with hot water, this method is difficult and hazardous to use and often reduces germination of the seed. Resistant varieties offer the best practical means of control.

Shands and Schaller (15) reported the reaction of a number of spring barley varieties and listed several with a high type of resistance. Poehlman (7) found good resistance in several winter barleys. Physiological specialization has been shown to occur within the loose smut organism (6, 11, 12), making it desirable to have more than one available source of resistance.

Equally important in a breeding program is an understanding of how resistance is inherited. Resistance controlled by a single gene is more readily utilized than that of a complex nature. A favorable linkage of resistance with some agronomically desirable character would greatly facilitate the breeding of resistant varieties. Shands (14) reported a linkage between the genes conditioning the loose smut resistance of Trebi and the stem rust resistance of Chevron. Selections are now available which carry these genes in the coupling phase. Early generation testing for loose smut resistance can be reduced by selecting stem rust resistant segregates.

Shortly after the present investigation was started, Livingston (5) reported that the resistance of Trebi was conditioned by a single gene. He found a similar gene in a selection of *Hordeum deficiens* (*Hordeum distichum* var. *deficiens* Steud.), and a weak gene in Missouri Early Beardless. There was no evidence of linkage between the genes for resistance and those for hoods or kernel row number.

In a series of crosses, Zeiner (16) found indications that single genes conditioned immunity, resistance, and moderate susceptibility. Resistance appeared to be dominant over susceptibility. The genes for resistance and moderate susceptibility were shown, in one cross, to be inherited independently. While not conclusive, Nahmmacher's work (6) suggested single gene differences between resistant and highly susceptible and between resistant and moderately susceptible varieties.

This paper deals with the genetics of resistance to loose smut in four varieties of barley. Varieties were selected which differed in their place of origin and in their level of resistance. These were crossed with susceptible varieties and, with one exception, in all possible combinations. Whenever the parents differed in morphological characters, the progenies were classified and tests of associations with resistance made.

MATERIALS AND METHODS

Hybrid populations obtained from crosses with six barley varieties were used in this study. The loose smut reactions of these varieties, as shown in table 1, varied over a wide range. Newal and Colsess IV were highly sus-

TABLE 1.—*Reactions of parental varieties when inoculated artificially with Ustilago nuda*

Variety	C.I No.[a]	Years tested	Tests[b]	Plants	Average infection
		No.	No	No.	Pct.
Newal	6088	5	30	1584	70.5
Trebi	936	5	31	1323	1.3
Colsess IV	5979	2	21	1092	84.9
Jet	967	2	16	299	0.0
X173-10 5 6 1		2	17	1196	43.6
Dorsett	4821	3	23	1275	12.9

[a] C.I. denotes accession number of the Division of Cereal Crops and Diseases Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture.
[b] A test represents the progenies from 2 inoculated heads.

ceptible, and selection X173-10-5-6-1 was moderately susceptible under the conditions of these tests. Jet, in which no infected plants have been found, and Trebi were highly resistant. Dorsett, with an average infection of 12.9 per cent, was considered resistant, but lacked the high type of resistance shown by Jet and Trebi.

Newal and selection X173-10-5-6-1 are white, 6-rowed, smooth-awned varieties, the former of commercial importance in Canada, the latter a Wisconsin selection from the cross [Oderbrucker (C.I. 4666) × Lion (Wis. 117)] × Oderbrucker (C.I. 4666). Trebi, a variety of commercial importance, has been used as a source of resistance to loose smut in many breeding programs. Colsess IV, developed by Robertson (8), carries the lethal seedling character xantha, a marker for linkage group VI, in a heterozygous condition. Both the heterozygous and homozygous green lines of this

stock were used.   Jet is a black, naked-kerneled, 2-rowed variety of Abyssinian origin, and Dorsett is a black, 6-rowed variety from Manchuria.

The "needle" method of inoculation, as described by Shands and Schaller (15), was used throughout this investigation.   A small rubber bulb containing dry chlamydospores was attached to a hypodermic needle (20-gage).   Using the tip of the needle to pierce the lemma of each flower on the spike, the dry spores were introduced directly into the flower by a gentle pressure on the bulb.   The small flowers at the terminal and basal joints of the rachis were removed before inoculation.   The inoculations were made in the field, except in a few cases where the F_1 generation was inoculated in the greenhouse.   Inoculations 1 day after anthesis of the median florets gave the most satisfactory results.   The plants from the inoculated seed were grown either in the field or in the greenhouse.   The progeny of each inoculated head was handled as a separate unit.

The inoculum was collected originally from a single smutted head occurring naturally in the susceptible variety Newal, on which it was increased and maintained.   The spores were freshly gathered, except in a few cases where they had been stored 2 or 3 months at $4°$ C.   In all cases a representative sample of the spores was germinated to determine their vigor and to ascertain the purity of the species.

Hybrid material was tested in the F_1, F_2, F_3, F_4, and backcross generations.   Since some genetically susceptible plants may escape infection, the F_2 does not permit a satisfactory genetical analysis.   Classification of F_2 plants, based on the behavior of their progenies in F_3 rows, and the F_2 progeny tests of backcrossed plants formed the basis for the genetical interpretation of the genes involved.   Limited tests of F_4 lines were made to check on the F_3 classification.

Because loose smut is floral-infecting, it is necessary to introduce the spores into the flowers of the plants producing the embryos of the generation to be tested.   The reaction of the F_1 generation was obtained by inoculating the flowers of the female parent 24 hr. after the introduction of the pollen from the male parent and then growing the plants from the inoculated seed.   Tests on the F_3 lines were made by inoculating the flowers of three heads of each F_2 plant taken at random from disease-free F_2 populations.   The three heads from each plant were kept separate and grown in individual rows representing three replications.   The average infection of the three rows was used in determining the reaction of the F_2 plant.   Since the plants varied in time of flowering, all inoculations could not be made on the same day.   Flowering time extended over a period of 10 to 14 days.   An analysis of variance on the percentage of infection in the parental varieties inoculated over the 10- to 14-day period showed that the variation in infection was not significant.   All F_3 lines of one replication were grown at the same time, usually one replication in the greenhouse and two in the field, except in a few cases where all three replica-

tions were grown in the greenhouse. Parental checks were included at regular intervals.

The material from the backcross was handled in a similar manner; *i.e.*, flowers on three heads of each backcross F_1 plant were inoculated. The F_4 tests were made by inoculating the flowers of a single head from a few plants in the noninoculated F_3 lines.

At harvest time, the plants were classified as either healthy or smutted. Plants with only one smutted tiller were classified as susceptible. The total number of plants and the number of smutted plants were recorded and the percentage of smut calculated.

Whenever parents of a cross differed in one or more morphological characters, segregations of these characters were observed. Classification was made on F_2 plants and, in general, verified in the F_3. Backcross plants and their progeny were classified also. Of particular interest was the association or lack of association of the morphological characters with smut reaction.

EXPERIMENTAL RESULTS

Smut Infection in the F_1 and F_2 Generations

Since infection occurs at flowering time, the infecting hyphae in order to reach the embryo must penetrate through floral tissues which may differ genetically from that of the embryo tested. It was necessary, therefore, to ascertain the effect of this maternal tissue on infection, and also whether resistance is determined by the genetic constitution of the floral tissue or that of the developing embryo.

TABLE 2.—*Reactions of the F_1 and F_2 generations of various barley crosses and their reciprocals when inoculated with Ustilago nuda*

Cross	Generation	Total plants	Infected plants	
		No.	*No.*	*Pct.*
Newal × Trebi	F_1	53	1	1.9
Trebi × Newal	F_1	61	0	0.0
Newal × Trebi	F_2	404	34	8.4
Trebi × Newal	F_2	121	8	6.6
Colsess IV × Jet	F_1	56	0	0.0
Jet × Colsess IV	F_1	28	0	0.0
Colsess IV × Jet	F_2	281	35	12.4
X173-10-5-6-1 × Dorsett	F_1	25	9	36.0
X173-10-5-6-1 × Dorsett	F_2	266	132	49.6
Dorsett × X173-10-5-6-1	F_2	138	64	46.4
X173-10-5-6-1 × Newal	F_2	195	125	64.1

The smut reactions of the F_1 and F_2 generations of the various crosses and their reciprocals are given in table 2. The amount of infection for the reciprocal crosses was the same. The 1.9 per cent infection in the F_1 of Newal × Trebi was no greater than would be expected for the resistant parent Trebi (Table 1). The lack of infection in the F_1 generation of

the crosses Newal × Trebi and Colsess IV × Jet must be attributed to the resistance of the heterozygous embryos, as the floral tissue of the female parents, Newal and Colsess IV, was susceptible.

The infection in the F_2 generations of the same crosses and their reciprocals (Table 2) showed that the fungus was able to become established in F_2 embryos which were surrounded by maternal tissue having the F_1 complement of genes for resistance and susceptibility. In this case, the embryos were surrounded by maternal tissue which was heterozygous for resistance. As was shown by the lack of infection in the F_1 generation, the heterozygous condition of the embryo gave the developing plant complete protection.
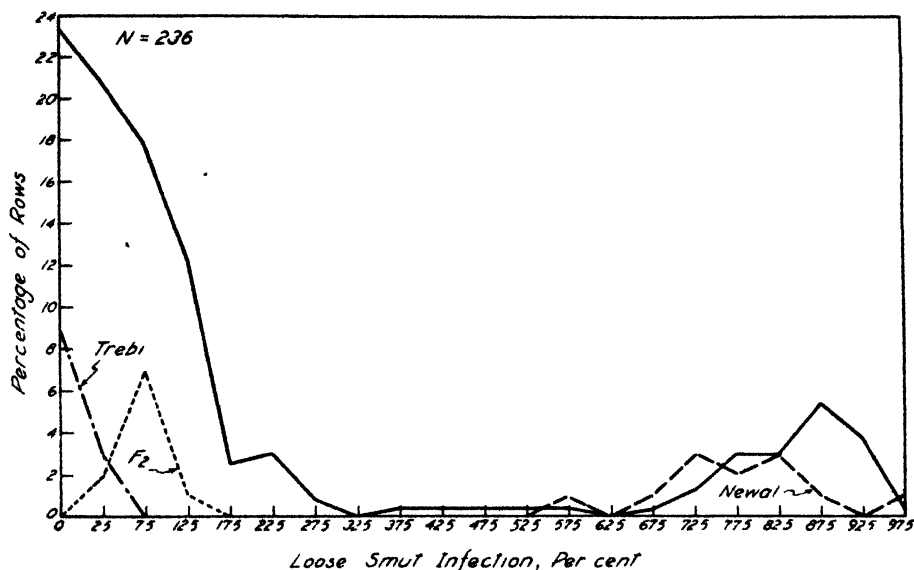
FIG. 1. Newal × Trebi. Distribution of parents, F_2 tests, and 236 F_3 lines in percentage infection classes. Parental and F_3 distributions are based on the number of tests rather than the percentage of rows in each infection class.

Lang (4) and Ruttle (12) have demonstrated that the barley loose smut fungus is able to reach the embryo by two different routes. The infecting hyphae can either follow the same route as the pollen tube or penetrate directly through the ovary wall. It is conceivable that resistant floral tissue might block direct penetration through the ovary wall, which would reduce the amount of infection in the F_2 generation. As will be pointed out later, the infection in the F_2 generation was slightly lower than that expected. There is a need for histological investigations on the mode of penetration when the floral tissue is resistant.

Inheritance of Resistance to Loose Smut in Hybrids with Trebi

Trebi was highly resistant and Newal highly susceptible when inoculated artificially. The smut reactions of the F_1 and F_2 generations (Table

TABLE 3.—*Distribution of parent rows, F₃, and backcross progenies for loose smut infection by percentage classes for crosses indicated*

Parent or cross	Percentage classes																					Total number of rows
	0	0.1–5	5–10[a]	10–15	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	85–90	90–95	95–100	
1943																						
Newal	9												1	0	1	3	2	3	1	0	1	12
Trebi		3				2	0	0	1	1	1	1	1									12
Newal × Trebi	55	49	42	29	6	7	2	0	1	1	1	1	1	0	1	3	7	7	13	9	1	236
1945 and 1946																						
Newal						2		0	1	0	1	2	0	1	2	0	0	1	0	0	2	10
Trebi		3	1			2																8
F₁ (Newal × Trebi) × Newal	3	7	10	4	2	0	0	0	2	2	2	1	3	1	3	3	1	4	3	4	2	57
1946																						
Coisess IV	7												1				2	3	1	3	2	11
Jet		6	13							0	0	1		1	1			3	1	0	2	7
Coisess IV × Jet	27	6	7	8	11	2	1	0	0	0	0	1	2	1	1	3	3	3	1	0	2	91
Coisess IV × F₁ (Coisess IV × Jet)		6	6	13	13	7	4	2	1	1	0	3	3	4	7	11	6	13	7	5	5	116
Trebi × Jet	56	15	3	1	2	2	0	0	0	0	1	0	1	0	1							89
1943																						
X173-10-5-6-1						1	3	2	2	2	2	1	1									12
Dorsett		2	2	3	3	1	1															12
X173-10-5-6-1 × Dorsett	1	9	18	17	9	11	13	21	19	21	22	12	16	10	8	2	3	1				231
1946																						
Dorsett × Trebi	43	21	7	0	2	1	0	2	0	4	4	2	4	1	0	2	1	1	1	1	1	96
Dorsett × Jet	46	17	13	9	1	2	2	2	2	2	0	1	0	0	2	0	0	1	0	1	0	99

[a] 5.1 to 10.0 per cent inclusive.

2) indicated that the resistance of Trebi was dominant.   No significant differences appeared between progenies of reciprocal crosses.

The smut infection of the parental varieties and 236 F_3 progenies by 5 per cent classes is given in table 3 and shown graphically in figure 1. The small number of lines between the 32 and 62 per cent infection classes divided the progenies into two groups, approximating a 3:1 ratio.   Such a ratio would be expected if the resistance of Trebi was conditioned by a single gene.

In order to separate accurately the homozygous susceptible and the segregating lines, the F_3 lines with an average infection between 28 and 58 per cent were tested in the F_4 generation.   Corresponding F_3 lines from noninoculated F_2 heads were used.   The results, given in table 4, showed

TABLE 4.—*Comparison of percentage smut infection of F_3 lines intermediate in loose smut reaction with percentage infection in corresponding F_4 lines.   Newal × Trebi*

F_2 plant number	Infection in F_3 generation	F_4 generation		
		Lines tested	Infection	
			Average	Range
	Pct.	*No.*	*Pct.*	*Pct*
12	28	4	4	0–8
24	29	4	6	0–11
126	30	3	6	0–11
40	39	4	49	30–58
123	40	4	82	62–95
155	48	5	79	68–93
96	51	3	76	67–90
125	58	4	66	42–88

that the F_3 lines with an average infection of 30 per cent or less were segregating for resistance, while those F_3 lines which had more than 30 per cent were homozygous for susceptibility.   The average infection of all F_4 lines of the latter group was 70 per cent.   Failure to obtain susceptible F_4 lines from the 3 segregating F_3 lines can be attributed to the small number of F_4 lines tested.

There were 190 F_3 lines with less than 30 per cent and 46 lines with more than 30 per cent infection.   Although the infection range of the 46 susceptible lines was slightly greater than that for the susceptible parent (Fig. 1), the F_4 tests showed them to be homozygous for susceptibility. The average infection for the 46 lines was 80.7 per cent, compared with 77.5 per cent for Newal.   The χ^2 test for goodness of fit to a 3 : 1 ratio gave a probability of 0.10 to 0.05.

No attempt was made to separate the homozygous resistant lines from those segregating for resistance, as considerable overlapping of the two groups would be expected on the basis of the distribution of the resistant parent and the F_2 tests (Fig. 1).   Since resistance was conditioned by a single gene, the distribution of the segregating F_3 lines should approximate

that of the F_2. The distribution of the parental checks and the F_2 tests shown in figure 1 and subsequent figures are based on actual test numbers rather than on percentage of rows since their infection extended over only a small portion of the curve.

The plants from backcross of F_1 plants to the susceptible variety Newal were grown without inoculation, and the lines obtained tested for smut reaction. Results of the reciprocal crosses, the F_1 used either as the male or female parent, were combined because of their similarity. The distribution of the 57 backcross F_2 lines is given in table 3 and shown graphically in figure 2. A complete separation of the lines into two groups occurred at the 25 to 40 per cent infection level.

There were 28 lines in the low infection group and 29 lines in the high infection group which conformed with the 1:1 ratio expected when one gene conditions resistance. As expected, the distribution of the lines in
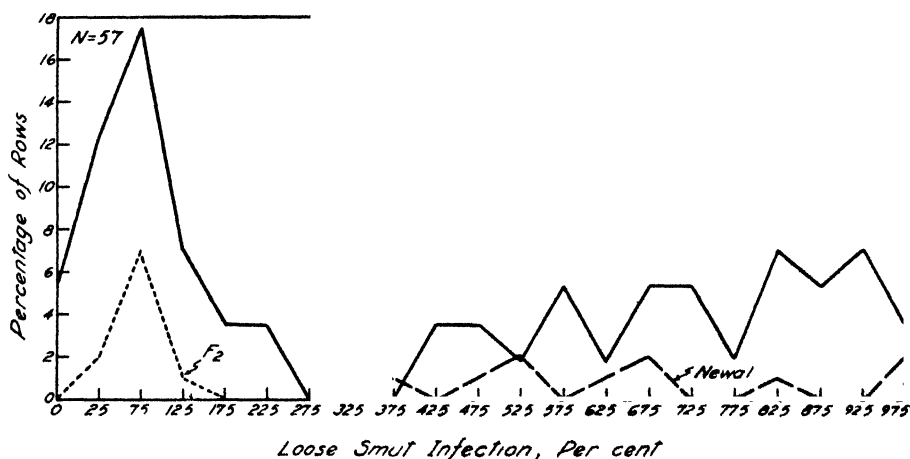
FIG. 2. F_1 (Newal × Trebi) × Newal. Distribution of the susceptible parent, F_2 tests, and 57 backcross lines in percentage infection classes. Parental and F_2 distributions are based on the number of tests rather than the percentage of rows in each infection class.

the low infection group conformed with the distribution of the F_2 tests (Fig. 2). The average infection of the F_2 tests was 7.5 per cent and for the 28 segregating lines 7.9 per cent. This was in close agreement, even though they were grown in different years. The spread of the lines in the high infection group was within the range of the susceptible variety (Fig. 2), and they can be considered homozygous for susceptibility. The average infection of this group was 73.8 per cent, for the Newal parent, 66.7 per cent. The wide range of infection in Newal and in the susceptible lines can be attributed to the fact that two years' results were combined. The level of infection for the two years differed considerably.

The results for the several generations of the cross Newal × Trebi and the backcross agreed very well with the ratios that would be expected if resistance of Trebi was conditioned by a single dominant gene. However,

one would normally expect a higher level of infection in the F_2 generation and in the F_3 rows from heterozygous F_2 plants. Part of this reduction can be accounted for by a slight selective mortality of infected plants caused either by low germination or weak germination followed by death of the seedlings. The F_3 families containing infected plants gave a lower percentage stand than those with no infection.

Infection groups	0	1.0 – 30	30.1 – 100
Average per cent stand	63	58	57

Selective mortality was also noted in the backcross lines.

Infection groups	1 – 30	30.1 – 100
Average per cent stand	58	52

Also, as was pointed out previously, the presence of resistant maternal tissue surrounding the developing embryos in heterozygous F_2 plants might possibly cause a slight reduction in infected plants.

Inheritance of Resistance to Loose Smut in Hybrids with Jet

Jet has maintained its high resistance throughout this investigation; no smutted plants appeared in any of the tests. Colsess IV was highly susceptible, averaging 84.9 per cent smutted plants. The F_1 plants were resistant when either variety was used as the female parent (Table 2). The absence of infection in the F_1 generation and the low amount in the F_2 generation suggested that the resistance of Jet was transmitted as a dominant character, and that the infection of the hybrids was determined by the nature of the embryo rather than by the floral tissue of the female parent.

The distributions of the parental varieties and 91 F_3 progenies for smut infection are given in table 3 and shown graphically in figure 3. The complete break in the curve at the 42.5 per cent infection class divided the progenies into two groups, 75 progenies with less than 37.5 per cent infection, and 16 with more than 47.5 per cent infected plants. This distribution suggested a 3 : 1 ratio, which would be expected if a single major gene for resistance was acting. The χ^2 test gave a probability of 0.20–0.10 for this ratio.

Since Jet was completely resistant, it can be assumed that the F_3 progeny rows from homozygous resistant F_2 plants would fall in the 0 per cent infection class. On the basis of the distribution of the F_3 tests (Fig. 3) and the distribution of the heterozygous backcross lines which will be discussed presently, very little overlapping of the homozygous and heterozygous F_3 rows would be expected. Considering the F_3 progenies with 0 per cent infection as homozygous resistant, those with infection up to 37.5 per cent as segregating, and those with more than 47.5 per cent as homozygous susceptible, the distribution fitted a 1 : 2 : 1 ratio with a probability of, 0.50–0.20.
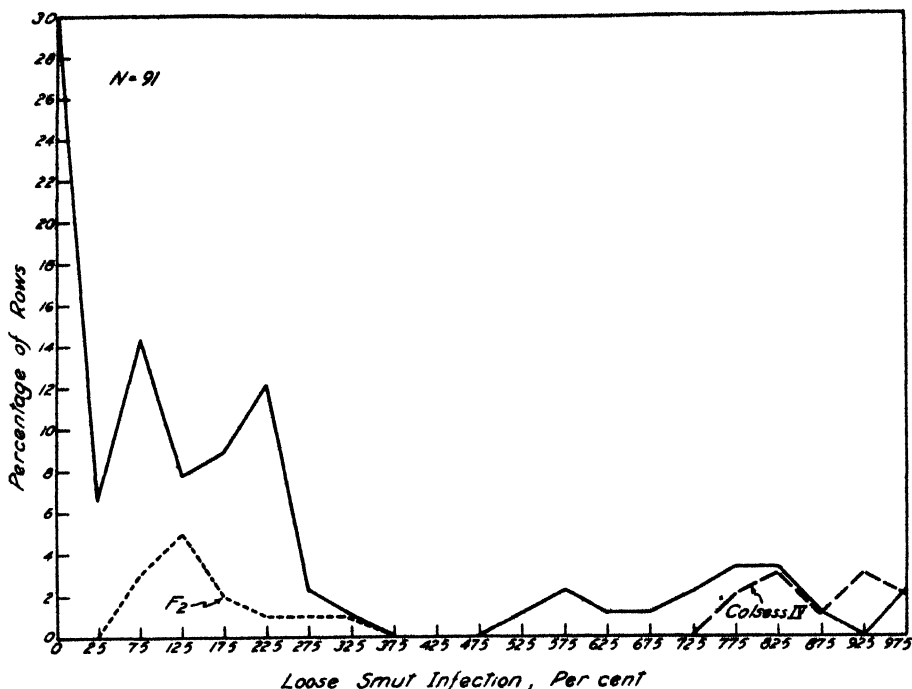
FIG. 3. Colsess IV × Jet. Distribution of Colsess IV, F₂ tests, and 91 F₃ lines in percentage infection classes. Parental and F₂ distribution are based on the number of tests rather than the percentage of rows in each infection class.

The distribution of 116 backcross F₂ progenies is given in table 3, and shown graphically in figure 4. The recurrent variety, Colsess IV, was used as the female parent. A complete break in the curve at the 47.5 per cent infection level separated the rows into two groups of approximately equal numbers. There were 52 lines with less than 47.5 per cent and 64 rows with more than 47.5 per cent infection. This suggested a 1:1 ratio
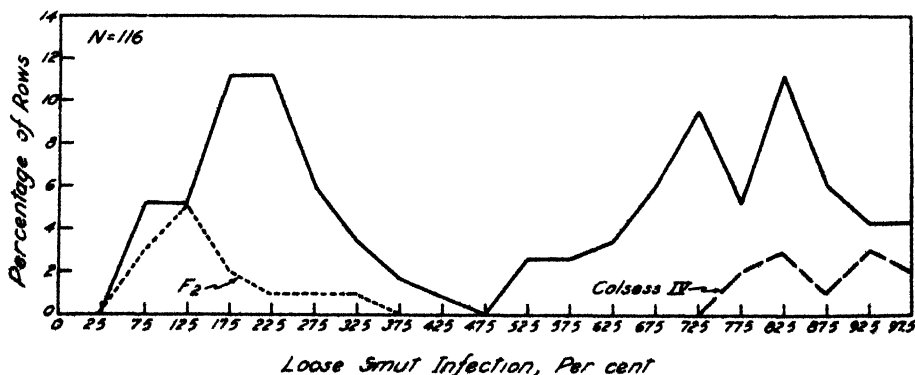
FIG. 4. Colsess IV × F₁ (Colsess IV × Jet). Distribution of Colsess IV, F₂ tests, and 116 backcross lines in percentage infection classes. Parental and F₂ distributions are based on the number of tests rather than the percentage of rows in each infection class.

of heterozygous and susceptible progenies, which would be obtained if a single dominant gene for resistance was present in Jet. The χ^2 test gave a probability of 0.50 to 0.30 for this ratio. The segregating rows averaged 20.8 per cent infection and the susceptible rows 80.0 per cent.

The various generations of the cross Colsess IV and Jet and the backcross gave distributions which agreed with the ratios expected if the resistance of Jet was conditioned by a single dominant gene.

The distribution of 89 F_3 progenies of the cross Trebi × Jet is given in table 3. Three rows had more than 45.0 per cent infection and were classed as susceptible. Since segregation occurred, the genes for resistance in Trebi and Jet were different. With two independent genes, approximately 1/16 of the population should be susceptible, or 5 rows from an F_3 population of 89 rows. The presence of 3 susceptible rows was within the range expected for independence, giving a probability of 0.50–0.30 with the χ^2 test. However, with such low numbers the χ^2 test is of limited value. If the genes were on the same chromosome pair, it would require a crossover value of about 38 per cent to obtain a total of three susceptible rows in the above population since they entered the cross in the repulsion phase. Independence of the two genes was suggested in unpublished data involving the crosses Jet × Brachytic, C.I. 6572, and Trebi × Brachytic. There was evidence of close association between the Jet gene for resistance and the gene or genes for loose smut resistance in Brachytic. No such association was noted in the Trebi × Brachytic cross.

Inheritance of Resistance to Loose Smut in Hybrids with Dorsett

Dorsett was moderately resistant and selection X173-10-5-6-1 moderately susceptible under artificial inoculation, with 12.9 and 43.6 per cent infected plants, respectively. The reactions of the F_1 and F_2 generations of the cross X173-10-5-6-1 × Dorsett are shown in table 2. The infection in the F_1 generation was slightly less than the average for the susceptible variety and the infection of the F_2 exceeded it. This increased infection in the F_2 suggested that selection X173-10-5-6-1 possessed a gene or genes for intermediate smut reaction. These data were of importance as an aid in interpreting the distribution of the F_3 progeny. The infection in the F_1 generation was an estimate of the infection which occurred in plants heterozygous for the factors for resistance, or, by the same reasoning, the amount of protection the factors in the heterozygous condition gave the plants. The infection in the F_2 generation was an estimate of the amount of infection expected in F_3 rows from F_2 plants which were heterozygous for the resistant genes.

The distribution of parental and F_3 progeny rows is given in table 3 and shown graphically in figure 5. Transgressive segregation toward the susceptible side was apparent; several F_3 progeny rows were more suscep-tible than selection X173-10-5-6-1. Evidence, to be presented later, sug-

gested that some of the progeny rows were more resistant than Dorsett, although the increased resistance was not great.

Progenies from eleven noninoculated F_3 rows which had more than 63 per cent infection in the inoculated F_3 complement were tested in the F_4 generation. The results are given in table 5. The average infection for all tests of the eleven progenies was 66 per cent, compared with 36 per cent for selection X173-10-5-6-1. The infection level of the checks was lower than when the F_3 progenies were grown, which would account for the lower infection in the F_4 generation compared with the F_3 generation (Table 5). The data on F_4 tests of progenies from comparable F_3 rows which had less than 12 per cent infection in the F_3 are given in table 5. The average infection for all tests of the 14 rows was 6 per cent, compared with 12 per cent for Dorsett. These results suggested a segregation for slightly increased resistance.
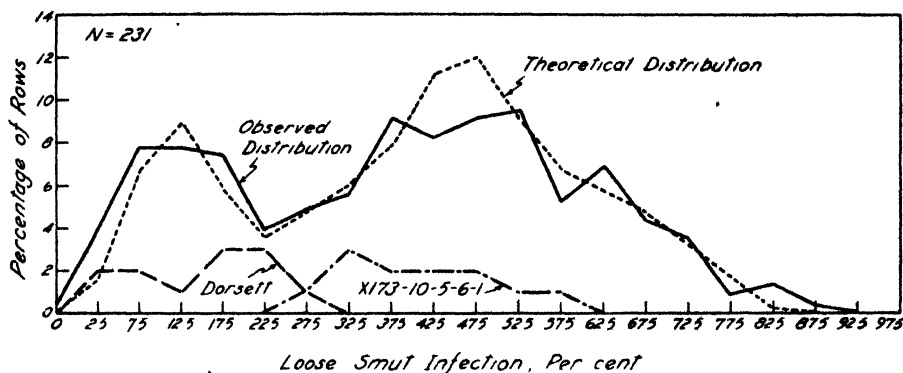
FIG. 5. X173-10-5-6 1 × Dorsett. Observed distribution of 231 F_3 lines in percentage infection classes compared with the theoretical distribution. Parental distributions are based on the number of tests rather than the percentage of rows in each infection class.

The bimodal curve given by the distribution of the F_3 lines suggested that resistance was controlled by relatively few genes. The hypothesis that each parent possessed a single gene for resistance satisfactorily explained the observed distribution. For convenience the genes contributed by Dorsett and selection X173-10-5-6-1 will be designated as D and X, respectively.

An attempt was made to assign infection values, consistent with known facts, to the F_3 rows representing the 9 different genotype combinations obtainable in a dihybrid ratio. On the basis of the parental reactions, the D and X genes permit approximately 13 per cent and 44 per cent infected plants, respectively, when in the homozygous condition. F_3 rows from F_2 plants homozygous for the parental gene combinations would be expected to have similar infection percentages. The F_3 lines from DDXX and DDXx plants should have as much or more resistance than Dorsett. This

was confirmed by F_4 tests. The lines homozygous for X and segregating for D, DdXX, would fall between the reactions of the two parents. The homozygous recessive lines, ddxx, would be completely susceptible.

The F_2 data (Table 2) indicated that F_3 rows from DdXx and ddXx plants should have an average infection of 48 and 64 per cent, respectively. As the D gene was more potent in conferring resistance than the X gene, lines homozygous recessive for X and segregating for the D gene, Ddxx, should fall somewhere between the values given above, namely 48 and 64 per cent. A value of 52.5 per cent was assigned as it met the requirements of the observed distribution. This value was substantiated by evidence in crosses of Dorsett with Trebi and Jet, which will be presented later.

The F_2 genotypes and the percentage of smut expected in F_3 rows are given below.

Ratio of F_2 genotypes		Mean percentage of smut in F_3 rows	Ratio of F_2 genotypes		Mean percentage of smut in F_3 rows
1	DDXX	7.5	2	Ddxx	52.5
2	DDXx	12.5	1	ddXX	40.0
2	DdXX	30.0	2	ddXx	62.5
4	DdXx	45.0	1	ddxx	72.5
1	DDxx	17.5			

The various genotypes were assigned values which were multiples of 2.5 per cent. Although the interactions of the genes could not be calculated accurately, the assigned mean values were consistent with what might reasonably be expected. In case of known reactions, such as parental types, the assigned values were within one standard deviation of the observed values.

Frequency distributions were calculated for the genotypes by using the means indicated and the standard errors derived from the formula $S = \sqrt{\dfrac{p\,q}{n}}$.

While this probably underestimated the errors since no account is taken of environmental effects, it gave a distribution which comformed very well to that observed. The separate distributions for the genotypes are shown in table 6. The combined values of the separate distributions gave the number of rows expected for the various classes. The theoretical distribution is given in table 6 and shown graphically by the broken line in figure 5. The agreement between the observed and theoretical distributions was good; the χ^2 test gave a probability of 0.95 to 0.50.

Dorsett was crossed with the resistant varieties Trebi and Jet, each of which contained a gene for resistance. Table 3 gives the distribution of the F_3 progeny rows. Highly susceptible rows occurred in both crosses, indicating that the genes for resistance in the three varieties were different.

When two genes are present, a ratio of 15 resistant and segregating to 1 susceptible would be expected. In the cross with Trebi, 6 lines had more

than 65 per cent infection and would be classified as susceptible. From the population of 96 F_3 rows, 6 were expected. Twelve lines had between 35 and 60 per cent infection, comprising about 2/16 of the population. On the basis of previous crosses, lines segregating for the Trebi gene would have less than 30 per cent infection. Consequently, these 12 lines would probably be segregating for the D gene and recessive for the Trebi gene.

TABLE 5.—*Comparison of percentage smut infection of low and high F_3 lines with percentage infection in corresponding F_4 lines. X173-10-5-6-1 × Dorsett*

F_3 plant number	Infection in F_3 generation	F_4 generation		
		Lines tested	Infection	
			Average	Range
	Pct.	*No.*	*Pct.*	*Pct.*
188	2	4	1	0–6
136	3	4	21	11–40
135	3	5	1	0–6
32	4	6	4	0–29
192	4	5	8	0–23
87	6	5	12	0–18
116	6	5	5	0–16
133	7	4	0	0
146	8	5	2	0–7
65	9	3	11	0–20
77	9	5	1	0–3
15	10	4	1	0–5
69	11	6	11	0–20
27	12	5	5	2–8
Av.	7		6	
127	63	3	33	32–33
79	67	3	89	83–100
13	69	3	48	30–80
196	70	2	96	93–100
61	71	5	34	7–64
194	72	5	59	22–86
44	72	5	69	50–100
11	73	4	50	35–68
211	74	5	71	40–91
105	76	4	70	50–90
78	81	4	67	67–72
79	67	3	89	83–100
13	69	3	48	30–81
196	70	2	96	93–100
Av.	71		66	
Dorsett		16	12	0–37
X173-10-5-6-1		16	36	22–60

The average infection of the 12 lines was 50 per cent which was in close agreement to the mean value of 52.5 per cent assigned to the lines segregating for D in the cross selection X173-10-5-6-1 × Dorsett.

While susceptible lines and lines with intermediate infection occurred in the cross Dorsett × Jet, there was a deficiency of these lines when compared with the number obtained in the cross Dorsett × Trebi (Table 3). In the latter cross there were 21 lines with 20 per cent or more infected plants,

TABLE 6.—*Theoretical distribution of F_2 rows of $X173$-10-5-6-$1 \times Dorsett$ for the genotypes indicated*

Infection classes	DDXX	DDXx	DDxx	DdXX	ddXX	DdXx	Ddxx	ddXx	ddxx	Rows expected	Rows observed	χ^2
Pct.										*No.*	*No.*	
0–5	3.04	0.65								3.69	10	3.8995
5–10[b]	8.35	6.67	0.61							15.63	18	0.2719
10–15	3.05	14.24	3.49							20.78	18	0.7948
15–20		6.67	6.24	0.79						13.70	17	0.0603
20–25		0.65	3.49	4.15						8.29	9	0.0046
25–30			0.61	9.50	0.53	0.14				10.78	11	0.0435
30–35				9.50	2.17	2.10				13.77	13	0.5673
35–40				4.15	4.52	8.77	0.38			17.82	21	1.6834
40–45				0.79	4.52	17.87	2.38			25.56	19	1.3609
45–50					2.17	17.87	6.72	0.31		27.07	21	0.0135
50–55					0.53	8.77	9.92	2.24		21.46	22	0.8972
55–60						2.10	6.72	6.86	0.08	15.76	12	0.4548
60–65						0.14	2.38	10.06	0.94	13.52	16	0.0512
65–70							0.38	6.86	3.50	10.74	10	0.0170
70–75								2.24	5.40	7.64	8	
75–80								0.31	3.50	3.81	2 ⎫	
80–85									0.94	0.94	3 ⎬	0.2336
85–90									0.08	0.08	1 ⎭	
90–95												
95–100												
Total	14.44	28.88	14.44	28.88	14.44	57.76	28.88	28.88	14.44	231.04	231	10.5035

ª Square of difference between number observed and number expected, divided by number expected.
b 5.1–10.0 per cent, inclusive.

compared with 13 lines in the Dorsett × Jet cross. If this difference was real, it would indicate a loose linkage between the D and J genes for resistance. Since the genes entered the cross in the repulsion phase, susceptible lines would result only from the union of crossover gametes, and lines with intermediate infection from the union of crossover gametes with noncrossovers. Consequently, a deficiency of these lines would be expected if the D and J genes were in the same linkage group. A crossover value of 34.6 per cent would be necessary to give the three susceptible rows that occurred.

While suggestive, the number of F_3 progeny tested was too small to establish definite linkage between the D and J genes. A crossover value of 34.6 per cent would give a distribution only slightly different from that expected from random assortment.

Association of Characters

In all of the crosses involving resistance *vs.* susceptibility, the parents differed in one or more morphological characters known to mark particular linkage groups. The markers used in this study have been found by several investigators to be controlled by single genes, and, with one exception, have been found to be inherited independently of each other. Daane (2) found the genes governing rough *vs.* smooth awns and long- *vs.* short-haired rachillas to be linked. A summary of these studies was reported by Robertson, Wiebe, and Immer (9), and therefore the individual references will not be cited here.

The variety Jet has a black lemma and pericarp, 2-rowed spikes, naked caryopsis, and full awns, and carries the allel for normal seedling color. In contrast, Colsess IV has a white lemma and pericarp, 6-rowed spikes, covered caryopsis, and hoods in place of awns, and carries the lethal seedling character, xantha, in the heterozygous condition. The genes conditioning these characters are located in linkage groups II, I, III, IV, and VI, respectively. Newal differs from Trebi in having smooth awns and long-haired rachillas. Both characters are markers for linkage group V. Dorsett is rough-awned, has a black lemma and pericarp and long-haired rachilla. Selection X173-10-5-6-1 has the allelmorph to each of these characters.

Classifications were made on F_2 plants and, in general, verified in F_3 lines. In the backcrosses, both the backcross plants and the progeny lines were classified. An attempt was made to verify previous reports as to the mode of inheritance of the genes, their relationships to each other and to the genes conditioning resistance to loose smut. Tests for goodness of fit and independence were made by means of χ^2. A P value greater than 0.05 was regarded as indicating satisfactory agreement.

The results of the χ^2 tests are summarized in table 7. With one exception, the segregation of the morphological characters fit monohybrid ratios, which is in agreement with published results. In the cross, Colsess IV × Jet, the segregation for non-6-rowed *vs.* 6-rowed spikes did not fit a 3:1

ratio because of an excess of 6-rowed segregates. However, in the backcross involving the same varieties, the normal 1:1 ratio was obtained for row

TABLE 7.—*Summary of χ^2 tests for mode of inheritance and relationship of characters in the various crosses*

Characters and crosses	Assumption	χ^2	D.F.	P
Newal × Trebi (N = 236)				
Rough *vs.* smooth awns (Rr)	3:1	0.08	1	0.80–0.70
Long- *vs.* short-haired rachilla (Ss)	3:1	1.19	1	0.30–0.20
Awns, rachilla hairs	9:3:3:1	13.70	3	< 0.01
Awns, rachilla hairs	Indep.	15.00	1	< 0.01
Smut infection, awns	Indep.	2.02	10	> 0.99
Smut infection, rachilla hairs	Indep.	5.85	10	0.90–0.80
F₁ (Newal × Trebi) × Newal (N = 57)				
Rough *vs.* smooth awns (Rr)	1:1	2.52	1	0.20–0.10
Smut infection, awns	Indep.	15.32	10	0.20–0.10
Colsess IV × Jet (N = 91)				
Black *vs.* white lemma and pericarp (Bb)	3:1	0.95	1	0.50–0.30
2-rowed *vs.* 6-rowed (Vv)	3:1	4.80	1	0.05–0.02
Hulled *vs.* naked ·(Nn)	3:1	0.05	1	0.90–0.80
Awns *vs.* hoods (Kk)	3:1	0.06	1	0.90–0.80
Lemma color, hull adherence	9:3:3:1	1.56	3	0.70–0.50
Lemma color, hoods	9:3:3:1	2.44	3	0.50–0.30
Hull adherence, hoods	9:3:3:1	0.53	3	0.95–0.90
Row number, hull adherence	Indep.	3.59	2	0.20–0.10
Row number, hoods	Indep.	5.85	2	0.10–0.05
Lemma color, row number	Indep.	5.79	2	0.10–0.05
Smut infection, lemma color	Indep.	5.42	6	0.50–0.30
Smut infection, row number	Indep.	1.63	6	0.95–0.90
Smut infection, hull adherence	Indep.	3.86	3	0.30–0.20
Smut infection, hoods	Indep.	9.63	6	0.20–0.10
Colsess IV × F₁ (Colsess IV × Jet) (N = 116)				
Black *vs.* white lemma and pericarp (Bb)	1:1	0.008	1	0.95–0.90
2-rowed *vs.* 6-rowed (Vv)	1:1	1.92	1	0.20–0.10
Awns *vs.* hoods (Kk)	1:1	0.42	1	0.70–0.50
Lemma color, row number	1:1:1:1	3.10	3	0.50–0.30
Lemma color, hoods	1:1:1:1	0.60	3	0.90–0.80
Row number, hoods	1:1:1:1	3.12	3	0.50–0.30
Xantha, lemma color	Indep.	1.20	1	0.30–0.20
Xantha, row number	Indep.	1.21	1	0.30–0.20
Smut infection, lemma color	Indep.	0.14	1	0.80–0.70
Smut infection, row number	Indep.	0.06	1	0.80–0.70
Smut infection, hoods	Indep.	0.14	1	0.80–0.70
Smut infection, Xantha	Indep.	0.00	1	> 0.99
X173-10-5-6-1 × Dorsett (N = 231)				
Rough *vs.* smooth awns (Rr)	3:1	0.64	1	0.50–0.30
Black *vs.* white lemma and pericarp (Bb)	3:1	0.24	1	0.70–0.50
Long- *vs.* short-haired rachilla (Ss)	3:1	0.64	1	0.50–0.30
Awns, lemma color	9:3:3:1	0.63	3	0.90–0.80
Awns, rachilla hairs	9:3:3:1	32.56	3	< 0.01
Awns, rachilla hairs	Indep.	28.30	1	< 0.01
Lemma color, rachilla hairs	9:3:3:1	1.13	3	0.80–0.70
Smut infection, awns	Indep.	6.79	10	0.80–0.70
Smut infection, lemma color	Indep.	6.98	10	0.80–0.70
Smut infection, rachilla hairs	Indep.	3.56	10	0.98–0.95

number. When selecting F₂ plants for inoculation, the only restriction imposed was that they produce sufficient seed for adequate F₃ tests. This

would tend to favor the 6-rowed types. Since there was no association be-
tween the genes conditioning smut reaction and row number (Table 7), the
differential selection of 6-rowed segregates would not affect the final an-
alysis of the genes involved in resistance.

The genes for rachilla hair length and awn barbing were found to be
linked, with a percentage recombination of 29 ± 3.1 and 29 ± 3.6, in the
crosses Newal × Trebi and selection X173-10-5-6-1 × Dorsett, respectively,
when computed according to the method of Immer (3). These percentages
are in agreement with those obtained by other investigators (9). All other
morphological characters showed independent segregation.

Complete independence of the genes conditioning smut reaction and the
various morphological characters was found in all crosses. Either the genes
conditioning smut reaction were located in linkage groups other than those
investigated or were so far removed from the marker genes that the linkage
was not detected. Markers for 5 of the 7 linkage groups were present in
the cross, Colsess IV × Jet, and the backcross, Colsess IV × F_1 (Colsess IV ×
Jet).

DISCUSSION

Four genes for resistance to loose smut of barley, *Ustilago nuda*, were
found in a series of crosses between susceptible × resistant and resistant × re-
sistant varieties. Resistance was found to be determined by the genetic
constitution of the developing embryo rather than that of the surrounding
floral tissue. The F_1 plants of the crosses Newal × Trebi and Colsess IV ×
Jet, with the susceptible variety as the female parent, were as resistant as
the resistant parent. In this case, the heterozygous embryos were sur-
rounded by susceptible floral tissue at the time of inoculation. The in-
creased infection in the F_2 generation of the same crosses and their recipro-
cals showed that the fungus was able to become established in F_2 embryos
which were surrounded by maternal tissue having the F_1 complement of
genes for resistance.

Reduced infection in the F_2 generation and in F_3 progeny lines from
heterozygous plants suggested a slight amount of interference by the resis-
tant maternal tissue in fungal penetration. This was noted by Livingston
(5) in studies with loose smut of barley and also by Caldwell and Compton
(1) in their studies on inheritance of resistance to loose smut in wheat.
However, reduced germination of infected seed, selective mortality of in-
fected plants, and any selectivity in the mortality of flowers after inocula-
tion would give similar reductions. A definite reduction in plant numbers
was noted in susceptible and segregating rows as compared with the resist-
ant rows in the cross Newal × Trebi and to a less extent in other crosses.
Although no measure was made of selectivity, the total mortality of inocu-
lated flowers was fairly great.

The genetical analysis of the Trebi crosses agreed with the findings of
Livingston (5), who reported a major gene for resistance in Trebi. This

gene was dominant in effect, the heterozygous plants being as resistant as Trebi, which occasionally had an infected plant. Robertson, Wiebe, and Shands (10) have assigned the symbol Un to this gene.

The high type of resistance in Jet was due to a single dominant gene. The heterozygous plants were as resistant as the parent. Jet was completely free of infection in all tests, both to the smut collection used in this study and to a composite of collections used in the breeding program. The occurrence of three susceptible F_3 lines in the cross Trebi × Jet indicated that the genes for resistance in Trebi and Jet were different. The population was too small to prove conclusively that these genes were in different linkage groups, but the data suggested independence.

The genetical analysis of the cross selection X173-10-5-6-1 × Dorsett was more difficult than for the previous crosses as more than one gene for resistance was present. Several F_3 lines were more susceptible than the susceptible parent. The presence of two independent genes for resistance, one contributed by each parent, satisfactorily explained the segregation in the F_3 rows. The genes in Dorsett and selection X173-10-5-6-1 differed from those in Trebi and Jet in that they permitted about 13 and 43 per cent of smut, respectively, when homozygous. The information available on the Dorsett and selection X173-10-5-6-1 genes indicated that they allowed 55 to 65 per cent smutted plants, respectively, when heterozygous. The individuals homozygous for both genes were slightly more resistant than Dorsett. The presence of the weak gene in selection X173-10-5-6-1 was substantiated further by the occurrence of 64 per cent infection in the F_2 generation of the cross Newal × X173-10-5-6-1, the parental varieties having an average infection of 70.5 and 43.6 per cent, respectively.

Segregation occurred in the crosses of Dorsett with Trebi and Jet. The Dorsett and Trebi genes were independent; the F_3 segregation gave the expected ratio of 15 resistant and segregating lines to one susceptible. Two-sixteenths of the lines had infection values ranging from 35 to 60 per cent, with an average infection of 50.0 per cent, which was in close agreement to the mean value of 52.5 per cent assigned to the lines segregating for the Dorsett gene in the cross selection X173-10-5-6-1 × Dorsett.

When the F_3 distribution of the crosses Dorsett × Jet and Dorsett × Trebi are compared, there is a noticeable deficiency of lines with more than 20 per cent infection in the former cross. Since the Trebi and Jet genes were similar in effect, parallel distributions would be expected if the genes were independent. A loose linkage between the Dorsett and Jet genes would account for this deficiency, as lines recessive for the Jet gene and either homozygous recessive or segregating for the Dorsett gene would result only from the recombination of genes from crossover gametes and the union of crossover with noncrossover gametes. Since the F_3 population was too small to establish the significance of this deficiency, it does not seem desir-

able to stress the possible linkage of the Dorsett and Jet genes until conclusive evidence is available.

Livingston (5) reported the presence of a weak gene in Missouri Early Beardless, which had an average infection of 53 per cent in his tests. However, this gene was ineffective against the smut collection used in the present study. In a number of tests with this collection, Missouri Early Beardless averaged 70 per cent infection and was classified as susceptible. Differences in varietal reaction showed that the weak gene in selection X173-10-5-6-1 was not the same as the one reported in Missouri Early Beardless.

While 4 genes for resistance were found, none occurred in combination. However, this might be expected as three of the varieties trace back to different places of origin, Trebi to Asiatic Turkey, Jet to Abyssinia, and Dorsett to Manchuria. It is expected that these genes would occur in other varieties originating in the same localities. Shands and Schaller (15) pointed out in their discussion that the intermediate type of resistance, as found in Dorsett, was common in the varieties from Manchuria, while those from Abyssinia had the highest proportion of varieties with the high type of resistance.

In keeping with the recommendations of Robertson, Wiebe, and Shands (10), the symbols Un_3, Un_4, and Un_5 are suggested for the genes in Jet, Dorsett, and selection X173-10-5-6-1, respectively. The symbols Un and Un_2 have been previously assigned to the Trebi gene and the weak gene in Missouri Early Beardless.

Independence of the genes conditioning loose smut reaction and the various morphological characters was found in all crosses. The Trebi gene for resistance was independent of those conditioning awn barbing and rachilla hair length, which are markers for linkage group V. Shands (14) reported a close linkage between the Trebi gene and the gene for stem rust resistance of Chevron, C.I. 1111, which is located in linkage group VII. A fairly close linkage was found between the Trebi gene and the gene for shortened internodes (Brbr), which is in group VII, in the cross Trebi × Brachytic, C.I. 6572 (unpublished data).

No association was found between the genes for resistance in selection X173-10-5-6-1 and Dorsett and those conditioning awn barbing, rachilla hair length, and lemma color.

Markers for six of the seven linkage groups were tested for their relationship to the Jet gene for smut resistance. Five linkage groups, I, II, III, IV, and VI, were involved in the cross Colsess IV × Jet and the backcross Colsess IV × F₁ (Colsess IV × Jet) (Table 7). The Trebi gene for smut resistance served as a marker for group VII in the cross Trebi × Jet. No evidence of linkage was detected with any of the six markers. If the Dorsett and Jet genes are linked as suggested earlier, independence of the Jet gene and the markers for linkage group V, awn barbing and rachilla hair length, can be assumed since the Dorsett gene was independent of

them. It is evident that the gene conditioning smut resistance in Jet was so far removed from the marker characters used in this investigation that linkage could not be detected.

SUMMARY

Evidence was presented which demonstrated the existence of four genes for resistance to loose smut of barley, three of which have not been reported previously. Each of the four varieties analyzed possessed a single gene which conferred its own degree of resistance. The genes in Trebi and Jet gave the highest type of resistance and were dominant in effect. The Dorsett and selection X173-10-5-6-1 genes allowed about 13 and 43 per cent of smutted plants when homozygous, and 55 and 65 per cent when heterozygous, respectively. The symbols Un_3, Un_4, and Un_5 were suggested for the genes in Jet and Dorsett and selection X173-10-5-6-1, respectively.

The Trebi gene was independent of the Jet and Dorsett genes. The Dorsett gene and the weak gene in selection X173-10-5-6-1 were also independent. Linkage between the Dorsett and Jet gene was suggested, but the evidence was not conclusive.

No associations were found between the genes for resistance and marker genes for the various linkage groups.

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PRODUCTION AND DISCHARGE OF BASIDIOSPORES BY PELLICULARIA FILAMENTOSA (PAT.) ROGERS ON HEVEA RUBBER[1]

J. B. CARPENTER[2]

A study of the production and discharge of basidiospores by *Pellicularia filamentosa* (Pat.) Rogers was made in connection with investigations on the Pellicularia leaf spot[3,4] of Hevea rubber trees, *Hevea brasiliensis* (H.B.K.) Muell.-Arg. and other species, in eastern Perú. In December 1947 an intensive search was made for the perfect stage of the fungus on infected leaves collected and examined during daylight hours. Although abundant hymenia were found, they had almost no basidia with either sterigmata or sterigmata and basidiospores, though some collapsed basidia were seen. As the fungus spreads rapidly under favorable conditions, it was assumed that it must sporulate heavily and frequently, if not in the daytime then perhaps at night. This paper describes the experiments and observations made during a study of the sporulation phenomena.

METHODS AND MATERIALS

The greater part of the work dealt with the behavior of the fungus on naturally-infected leaves bearing large lesions with an extensive hymenium, which appeared as a silvery bloom within the diseased area on the dorsal side of the leaf. The methods used for studying basidiospore discharge were modified after those employed by Keitt and Jones[5] and Keitt *et al.*[6]

A suitable portion of leaf, at least 3 × 5 cm., was removed and put into a moist chamber, which was usually a Petri dish cover. The ventral side of the leaf piece was pressed against a wet filter paper in the inside top of the chamber, without touching or wetting the hymenial surface. The

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chambers were arranged on a base covered with moist filter paper, so that each leaf piece was over a countersunk wooden drawslide, just wide enough to accommodate a glass microscope slide and long enough to permit the insertion and removal of slides. Thus, successive slides were exposed beneath the same leaf surface, without seriously disturbing the air within the chamber, or the chamber itself. The slides were coated lightly with glycerin jelly.

High humidity was maintained in the chambers; each was opened once daily for a few minutes to collapse the often abundant, sterile, aerial mycelium, and to permit moistening of the filter papers. The chambers were then replaced in their original positions.

Spore counts were made as follows. Cotton blue in lacto-phenol was placed on the slide and covered with a 50×24 mm. coverglass. Fifty well-distributed fields per slide were counted or estimated, using a mechanical stage. The results are expressed as the average number of basidiospores per sq. cm. per time interval.

Unless otherwise noted, the trials were made in a white-walled room with two large uncurtained windows on the east and west sides respectively, so that the chambers were in strong diffuse natural light during the day and normal darkness at night.

The fungus was also grown in pure culture on soil agar, where it produced a fertile hymenium within a few days on the sides of the culture vessel, which was usually a half-pint milk bottle. The agar was prepared as follows: A few grams of moist soil with a high content of organic matter and 3 or 4 wheat seeds were placed in each vessel, steamed for 30 min., and left overnight. The following day, water agar was added to a total depth of 1 cm. and the vessels were plugged, autoclaved for 45 min. at 15 lb. pressure, cooled in a horizontal position, inoculated, and incubated in a horizontal position at room temperature.

Also, hymenia were produced by transferring a 1×2 cm. portion of an active plate culture, preferably on deep soil agar, to a sterile Petri dish containing 1 or 2 cc. of water agar along one side of the plate to help maintain high humidity. The block was placed at some distance from it. Fertile hymenia were produced within 24 to 48 hr. on the glass and could be studied directly without being disturbed.

EXPERIMENTAL RESULTS

First to be studied were the daily fluctuations in basidiospore discharge under natural light conditions. The data given in table 1 were obtained from two trials made 9 months apart and are in·agreement with other simpler preliminary trials. Data on basidiospore discharge are presented by 6-hr. intervals, since detailed data for a 4-day period appear in table 2. It may be seen that the bulk of the spores were discharged during the

night, from 6 P.M. to 6 A.M., with a much lower rate of discharge during the daytime, especially on sunny days. All the hymenia studied gave relatively heavy discharges at night throughout the trial period, but many gave no discharge during a period of 6 to 12 hr. in the daytime.

The ability of the fungus to continue growth and active spore production on detached leaf pieces, over a period of several days, suggests that lesions on favorably disposed fallen leaves may continue to produce abundant inoculum for some time. During the initial search for the perfect stage, numerous well-developed active hymenia were found on such leaves.

The second phase of experimentation was a preliminary inquiry into the factors governing sporulation. In a 3-part experiment in February 1949, the material was subjected to (a) natural light, (b) prolonged light,

TABLE 1.—*Basidiospore discharge* *under natural light by Pellicularia filamentosa on infected leaves of Hevea brasiliensis*

Interval (in hours)	Trial A[b] day number					Trial B[c] day number			
	1	2	3	4	5	1	2	3	4
6 P.M. to midnight	207	1248	7953	8767	2989	675	15271	13078	13123
Midnight to 6 A.M.	179	8	3130	1855	535	2514	8338	9625	6713
6 A.M. to noon	T[d]	77	10	17	2[e]	108	143	305	254[e]
Noon to 6 P.M.	T	35	T	0		569	757	369	

[a] Basidiospore discharge expressed as average number of spores per cm.[2] based on counts of 50 fields per slide
[b] Begun April 13, 1948; one leaf only; bright sunny days.
[c] Begun January 12, 1949. One leaf only first day, average of 6 leaves thereafter; cloudy nearly all day every day.
[d] Trace, less than $\frac{1}{4}$ spore per cm.[2]
[e] Ended 8 A.M.

and (c) prolonged darkness, with the resumption of alternating light and dark periods in each (b) and (c) after 42 hr., which period had embraced two night and day cycles. Six leaf pieces were used in each part of the trial. Three pieces were cut from each of 6 leaves bearing extensive hymenia, and one piece was used in each part of the experiment, so that each "number 1" leaf piece had a common origin. Fortunately, all 6 leaves had fertile hymenia and it seems safe to assume that the principal variations among the treatments were due to environment and not to differences in the test materials.

The controlled light was furnished by a 100-watt Mazda lamp in a reflector, at 18 in. from the chambers. Darkness was obtained in a windowless closet.

No critical temperatures were encountered within the range of 21.5° to 28° C.; the temperature usually varied between 23° and 25° C.

The data from this experiment are presented in table 2. Basidiospore discharge under natural light conditions had a similar distribution to that

in the trials reported in table 1, with the heavy sporulation rate at night and a very low sporulation rate during the day, over a period of four night-and-day cycles. Under prolonged light, sporulation was retarded for about 6 hr. beyond the end of normal daylight, after which substantial sporulation occurred in two distinct cycles of 12 and 15 hr. each, separated by a 9-hr. interval of low sporulation. In prolonged darkness, heavy discharge occurred during the two normal night periods, but was low during the periods corresponding to daytime.

TABLE 2.—*Effect of various light conditions on basidiospore discharge* by Pellicularia filamentosa *on infected leaves of Hevea brasiliensis*

Day	Interval, in 3-hr. periods	Light conditions[b]		
		Natural	Controlled	
			Prolonged light	Prolonged darkness
1	6 P.M.	617	2	471
	9 P.M.	3645	16	4657
	Midnight	4376	416	3474
	3 A.M.	1052	370	658
	6 A.M.	66	671	23
	9 A.M.	0	920	50
	Noon	0	195	135
	3 P.M.	0	0	219
2	6 P.M.	2771	132	364
	9 P.M.	8018	1968	2318
	Midnight	4822	1367	5287
	3 A.M.	2262	994	4876
	6 A.M.	164	1150	2634
	9 A.M.	2	1024	938
	Noon	0	1798	163
	3 P.M.	2	5007	62
3	6 P.M.	4494	4388	730
	9 P.M.	10999	2008	5128
	Midnight			
	3 A.M.	1761	T[c]	7992
	6 A.M.	118	0	5586
	9 A.M.	0	0	4834
	Noon	0	0	2686
	3 P.M.	8	381	52
4	6 P.M.	3016	3663	5
	9 P.M.	5150	4990	17
	Midnight	2306	3836	305
	3 A.M.	719	417	2171
	6 A.M.	71	3	3850
	9 A.M.	0	End	3059
	Noon	End		1629
	3 P.M.			58
5	6 P.M.			0
	9 P.M.			End

[a] Basidiospore discharge expressed as average number of spores per cm.^{2}, based on counts of 50 fields per slide, using 6 leaf pieces in each series.

[b] Natural light indicates strong diffuse daylight and darkness in normal cycle; controlled light provided by a 100-watt Mazda lamp; controlled darkness obtained in a windowless closet. Boldface type indicates intervals of darkness. Under natural conditions, darkness prevails from about 7 P.M. to 6 A.M.

[c] Trace, less than 1 spore per cm.^{2}

When the controlled light series was returned to a sequence of typical light and dark periods, sporulation was similar to that found under natural light conditions, although the hymenia originally in prolonged darkness did not seem to readjust themselves as rapidly as those under prolonged light.

The third phase of the work dealt with a study of sporulation by the fungus when grown *in vitro* on soil agar. The slides, which were exposed beneath hymenia growing in half-pint milk bottles, were held by a wooden snap-clothespin, one arm of which was shortened so that the other could be inserted into a No. 8 rubber stopper, which replaced the cotton plug. Although an aseptic technique was not used, contamination offered no difficulties over a period of a few days.

Representative data are presented in table 3, indicating that sporulation in pure culture is similar to that encountered on naturally-infected leaves.

TABLE 3.—*Basidiospore discharge* by *Pellicularia filamentosa in vitro and under natural light*

Interval, in hours	Day number				
	1	2	3	4	5
8 P.M. to midnight	123	267	1732	2343	2309
Midnight to 6 A.M.	404	351	1271	1293	5779
6 A.M. to noon	4	T[b]	2	0	32
Noon to 6 P.M.	0	1	0	0	0

[a] Basidiospore discharge expressed as average number of spores per cm.^{2} based on counts of 50 fields per slide, for each of 5 replicates. Experiment begun on March 4, 1949.

[b] Trace, less than 1 spore per cm.^{2}

Using the Petri dish technique for producing hymenia *in vitro*, it was possible to observe microscopically the changes that took place in the fungus. In a typical example, the following microscopic observations were made under natural light conditions, observing the same area throughout. The fungus was illuminated for about 1 min. at each observation.

3 A.M. The basidia appeared as dense, almost spherical bodies grouped on short lateral hyphae.

5:30 A.M. A few basidia had short sterigmata.

9:10 P.M. There was abundant spore production, with typical mature basidiospores borne on long sterigmata. No actual discharge was seen, although occasional violent action was noted in some denser parts of the mycelium.

11 P.M. Similar to 9:10 P M.

5:30 A.M. There were almost no full-grown spores *in situ*, although some sterigmata still bore small, immature spores. Clearing and collapse of the spent basidia were beginning.

10 A.M. The spent basidia were distinctly collapsed and the immature basidia were still without sterigmata.

DISCUSSION

Basidiospore production and discharge in the *Hevea* strain of *Pellicularia filamentosa* appear to be a periodic function which may be strongly influenced by the presence or absence of light, at least over a period of a few days. Under natural light, the heaviest spore production and discharge occurred at night; sporulation in the daytime was low or absent. Since sporulation was sharply reduced even by diffuse daylight, it might be assumed that under the strong light found in the field daytime sporulation is negligible except in heavily shaded areas. No critical temperatures were encountered during the experiments. High humidity was favorable for the organism; no study was made on the effect of low humidities.

This fungus seems to be well adapted for persistence and for parasitizing the host, within its environment. The period of heavy sporulation coincides with the period of lower air temperature and increased humidity, commonly accompanied by a heavy dew formation that moistens the leaves of the host and provides conditions favorable for both the production and germination of basidiospores, since the temperature seldom falls below 16° C. The frequency with which the heaviest discharge occurs between 6 P.M. and midnight, or the first 6 hr. of darkness, appears to be more than a coincidence, since it would permit a long infection period for a maximum amount of inoculum.

The dew persists until after dawn in the Peruvian rubber-growing areas, and ground fogs frequently retard drying, even during the dry season. During the rainy season, night and early morning rains, and the prevailing high humidity, give conditions leading to severe disease development. Some new infections may always be found even in the dry season, when daytime temperatures are relatively high and the humidity is low, and there is little rainfall.

SUMMARY

Sporulation by the *Hevea* strain of *Pellicularia filamentosa* under natural light, both on infected leaves and *in vitro*, appears to be a periodic function, with the heaviest rate of basidiospore discharge occurring during the night and only a low to negligible rate of sporulation during the daytime.

Preliminary results suggest that periodic sporulation may be strongly influenced by manipulation of the light and dark periods to which the fungus is exposed.

The *Hevea* strain of *P. filamentosa* appears to be well adapted for persistence and for parasitizing the host within its environment.

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PROGRESS IN THE STUDY OF DWARF BUNT OF WINTER WHEAT IN THE PACIFIC NORTHWEST[1]

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Dwarf bunt (*Tilletia caries* (DC) Tul.) of winter wheat is widely prevalent in the Pacific Northwest[3] and has a more limited distribution in other regions (2, 11, 14). Because of certain characteristics (1, 5, 7, 8, 10, 15), some of which distinguish it from common bunt, dwarf bunt presents unique problems of investigation and control. Consequently, little is known about the nature of this disease and the biology of its causal fungus. In 1942 a series of studies was begun to elucidate some of the factors relating to the behavior and control of dwarf bunt. This paper constitutes a report on the progress of these studies.

MATERIALS AND METHODS

The scope of these investigations extended from field observations on factors relating to the occurrence of dwarf bunt to experimentation on the biology of the causal organism. Annual observations were made to determine the prevalence and severity of the disease under varying weather and soil conditions, and the tendency toward cyclic occurrence of epiphytotics. Observations were made on reactions of varieties in commercial and experimental plantings in different localities, both from the standpoint of resistance to dwarf bunt and that of the characteristics of diseased plants.

Nine collections of dwarf bunt were used in studies on physiologic specialization, chlamydospore germination, and nuclear behavior. Five of these collections were from Washington, two from Idaho, and one each from Montana and Utah. The spores of all collections were treated according to the method described previously (6), and inoculations were made by dipping the wheat kernels in a suspension of treated spores, after which the seed was dried before planting. All germination tests in the laboratory were made on 2 per cent water agar at approximately 8° C. Studies on nuclear behavior were made with material that was stained with Heiden-

hain's iron-alum haematoxylin. Other materials and methods will be described with the presentation of results.

Seed Inoculation

Failure to obtain infection from seed-borne chlamydospores of dwarf bunt is one of the major handicaps in the investigation of this disease. Within the last 15 years many routine inoculations have been made with dwarf bunt spores at experiment stations in the Pacific Northwest. Almost without exception the results from these tests have been negative. One exception worthy of note was recorded at Logan, Utah, in 1946. Seed of a susceptible variety (Turkey 926), naturally contaminated with dwarf bunt spores, was planted in irrigated soil and produced a crop with 25 per cent dwarf bunt. No dwarf bunt occurred in several other susceptible varieties included in this test and grown from noncontaminated seed. In this instance, therefore, an appreciable amount of infection was obtained from seed-borne inoculum. On the whole, however, experimental evidence and experience prove that this method of making dwarf bunt tests is not reliable.

The tests referred to above were made with chlamydospores as collected from the field. Additional tests were made with dwarf bunt spores which were given special treatment, including presoaking at low temperature, which is known to induce germination (6). For one test with spring wheat at Bozeman, Montana, in 1943, smutted heads of wheat were stored dry in the laboratory in the fall of 1942. In February 1943 some of this material was placed outside in the snow and allowed to remain until May, and the remainder was kept in the laboratory. Seed of two susceptible spring wheat varieties (Ulka, C.I. 11478, and Ceres, C.I. 6900) was inoculated with dwarf bunt spores of both treatments. No infection was obtained in this test.

In a test at Pullman, Washington, seed of several susceptible winter wheat varieties was inoculated with spores of 9 collections of dwarf bunt. These spores had been presoaked in water in a refrigerator for six months (6). The inoculated seed was planted in the field in October. No infection was obtained in any of the varieties.

Since dwarf bunt can seldom be reproduced by seed inoculation with chlamydospores, most of the information on its behavior has been obtained from field observations. During the course of this work special emphasis has been placed upon observations and studies of factors pertaining to the natural occurrence of the disease in commercial wheat fields. These have revealed certain phenomena relative to the incidence of dwarf bunt which may or may not have a bearing on final solution of the problem.

Perennial Nature of Soil Infestation

Dwarf bunt, being primarily a problem of soil infestation, cannot be controlled by seed treatment. It can be controlled by growing resistant varieties. Crop rotation has been considered as another possible means of control. However, successful control of any disease by this method depends upon how long the infestation from any one crop persists in the soil. That dwarf bunt persists for at least two seasons is suggested by the fact that there seems to be no correlation between the severity of the disease in one year and that in the succeeding year. Numerous observations in commercial fields have shown that heavy infestations frequently follow years of light infestation and vice versa.

Alternating years of light and heavy incidence of dwarf bunt appeared to be the rule in the infested areas of Montana. In the ten-year period, 1938–1948, records show that in the Gallatin Valley near Bozeman there was severe incidence of dwarf bunt in the even-numbered years, in contrast to relatively light incidence in the odd-numbered years. Fields with 75 per cent dwarf bunt were common in this area in the heavy-incidence years during the first half of this period, before resistant varieties became generally grown. By contrast, in the light-incidence years dwarf bunt occurred only in traces or small percentages. The only exception to this was in 1943 when some fields had spots up to an acre in size with as much as 50 per cent dwarf bunt.

The pattern of alternating years of light and heavy incidence was likewise typical of the Flathead Valley near Kalispell, Montana. There, however, the light and heavy infestations were in even and odd years, respectively, which is in the reverse order of that in the Gallatin Valley. The alternating pattern in the Flathead Valley was broken in 1946, when dwarf-bunt incidence was light.

In other areas, alternating years of light and heavy incidence of dwarf bunt are not the rule. Nevertheless, recorded observations in all areas of infestation show a definite lack of correlation between the amounts of dwarf bunt in successive years. For example, in southern Idaho and northern Utah the incidence of dwarf bunt was unusually severe in 1935; in many commercial fields estimated percentages of dwarf bunt were above 50 per cent and occasional ones had more than 75 per cent. Although this devastating incidence followed and was in turn followed by years (1934, 1936) of mild dwarf bunt, an alternating cycle was not evident in other years. In this area it has been observed that severe dwarf-bunt incidence usually follows early fall emergence of wheat, although at least one year (1946) was an exception to this rule.

There is some evidence that infective inoculum of dwarf bunt persists in the soil much longer than 2 years. For example, in old centers of infestation where resistant varieties have been grown for 7 to 10 years, thus

reducing to a minimum the amount of reinfestation, high incidence of dwarf bunt has been obtained in experimental plantings of susceptible varieties. In a commercial field in Utah which was at one time heavily infested and had been planted to resistant varieties for 7 years, as much as 65 per cent dwarf bunt occurred in the susceptible varieties of an experimental planting in 1946. Similar observations were made in Montana where two heavily infested fields in 1942 were grown to the resistant variety Wasatch in all succeeding crop years. The susceptible varieties in an experimental planting in these fields in 1948 averaged above 50 per cent dwarf bunt. The area surrounding these fields had likewise been relatively free of dwarf bunt for 4 years, owing to the use of resistant varieties. These observations suggest, therefore, that dwarf-bunt infestation of the soil may persist for at least 4 and possibly as long as 7 years.

Further evidence of the perennial survival of dwarf-bunt inoculum in the soil was obtained from an experiment[4] in which contaminated soil was protected from recontamination by wind-borne spores. Spore-proof boxes were used to cover plots that had been inoculated with dwarf bunt in the form of smutted heads of wheat, smut balls, and powdered spores. Each fall for three years the boxes were removed and the susceptible variety Utah Kanred was planted. Up to 20 per cent dwarf bunt occurred in these plots and there was no difference in the amount of infection where different kinds of inoculum were used. No dwarf bunt developed in the check plots, thus indicating that the boxes effectively protected the soil from contamination by wind-borne spores. This is additional evidence that dwarf bunt can persist in the soil for several years and for this reason it cannot be controlled by crop rotation.

Conditions Favoring Development of Dwarf Bunt

Little is known about the specific environmental requirements for the development of dwarf bunt. The nature of the disease and its causal agent has made it practically impossible to determine these requirements experimentally. Field observations during the last 15 years, which suggest certain conditions under which dwarf bunt develops most severely, are presented primarily as a basis for future study and observations.

Dwarf bunt occurs under a wide range of conditions in the Pacific Northwest. Early records indicate that it was most severe in low-rainfall, light-soil regions where the Turkey (Hard Red Winter) wheats predominate. More recently, however, it has reached destructive proportions in areas of higher rainfall and heavy soils where soft white and soft red winter wheats predominate. Furthermore, it occurs in irrigated winter wheat in the Gallatin Valley of Montana. Although there is no evidence that dwarf bunt has infested the irrigated soils of Cache Valley in Utah, it has

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[4] Planned and conducted by H. A. Rodenhiser in cooperation with R. W. Woodward.

been produced experimentally under irrigation at Logan. In this experiment irrigated plots were inoculated with 2 gm. of spores per square foot 5 days before seeding a nursery of 25 varieties. A duplicate nursery was seeded in naturally infested soil under dry-farm conditions. The percentages of dwarf bunt on 7 susceptible varieties in these two nurseries are presented in table 1. It is apparent from these data that dwarf bunt develops at least as well under irrigation as under dry-farm conditions. Thus, from the above observations and experimental data it is evident that dwarf bunt is widely adapted in its soil and moisture requirements for good development.

Although there is no indication that soil type is a critical factor in dwarf-bunt development, there is some evidence that soil condition has a pronounced influence in this respect. Recorded observations show that this

TABLE 1.—*The percentages of dwarf bunt produced in susceptible wheat varieties in artificially inoculated irrigated plots and in naturally contaminated dry-farm plots at Logan, Utah, in 1946*

| Variety | C.I. Number | Percentages of dwarf bunt in | |
		Irrigated plots	Dry-farm plots
Hybrid 128	4512	90	63
Ridit	6703	5	2
Yogo	8033	65	70
Rio	10061	75	37
Brevon	11912	63	6
Elgin	11755	80	75
Minturki	6155	17	5

disease has a tendency to occur with variable severity in different parts of the same field. Localized spots of severe infestation varying from a few square feet to an acre have been observed in all areas of infestation. This is particularly true in regions of new infestation. More frequently than not, infection centers of this type occur where the soil becomes excessively compact; for example, dwarf bunt has repeatedly been observed to be more severe along certain slopes and ravines and at turning corners, and in the drill rows over which the wheels pass in the seeding operation.

The effect of packing the soil on the development of dwarf bunt was tested experimentally at Bozeman, Montana. Seed of the dwarf-bunt-susceptible variety Newturk was sown in a commercial field known to be heavily infested with dwarf-bunt inoculum. The seed was planted in rows 20 ft. long and replicated four times. In half of the rows the soil was compacted over the seed by tamping, the other rows being left as checks. The average percentage of dwarf bunt in the tamped rows was 58 while in the check rows it was 24, thus indicating that a compact condition of the soil favors dwarf-bunt development.

Degree of Dwarfing

Dwarf bunt reduces the height of infected plants to a striking degree. The percentage of reduction in height was determined on six different varieties by measuring 100 culms each of smutted and nonsmutted plants in commercial wheat fields. Three varieties near Bozeman, Montana, and three other varieties in experimental plots near Logan, Utah, were measured. The data are presented table 2.

The average height of smutted plants in four fields of Turkey wheat ranged from 8.44 to 15.62 in. compared with 24.80 to 39.86 in. for nonsmutted plants. This represents a reduction in height ranging from 58 to 66 per cent, an average of about 63 per cent. The average reduction in plant height in three fields of Turkey in 1942 was 66 per cent. Smutted plants in one field of Cache were reduced in height by 56 per cent in 1943 (Table 2), and 59 per cent in 1942. A similar degree of reduction in

TABLE 2.—*Degree of height reduction caused by dwarf bunt in six varieties of winter wheat*

Field No.	Variety	Year	Average height in inches		Percentage reduction in height
			Smutted	Healthy	
1	Turkey	1943	13.74	39.86	66
2	Turkey	1943	9.92	27.12	63
3	Turkey	1943	8.84	24.80	66
4	Turkey	1944	15.62	37.08	58
5	Cache	1944	17.76	40.06	56
6	Tenmarq	1944	13.73	37.23	63
7	Tenmarq	1946	10.72	27.22	60
8	Triplet	1948	11.61	23.73	51
9	Utah Kanred	1948	11.27	26.19	57
10	Minhardi	1948	11.59	28.25	59

height was found in Utah Kanred and Minhardi. Height reduction in the variety Tenmarq was intermediate between that of Turkey and Cache (Table 2). Smutted Triplet plants were about one-half the height of healthy plants of that variety. In Turkey and Tenmarq the infected plants were about one-third the height of healthy plants. In the other varieties studied the reduction was somewhat less, the height of smutted plants being between one-half and one-third that of healthy plants. Thus it appears that degree of height reduction caused by dwarf bunt depends upon the variety infected.

The slight difference in degree of stunting in Turkey and Cache may be due to the tendency toward partial smutting in the latter variety. In Turkey, partially smutted plants and heads are a rarity while in Cache they occur frequently. Invariably, the smutted culms of a partially smutted plant of any variety are taller than those of a completely smutted

plant. Likewise, partially smutted heads are higher than completely smutted heads of the same plant. In a commercial field of Pawnee near Waterville, Washington, which had 15 per cent dwarf bunt, the partially smutted heads were one-third to one-half as high as the nonsmutted heads. In contrast, however, there was no partial smutting in Triplet, and infected plants of this variety exhibited the least degree of dwarfing.

Physiologic Specialization

Since dwarf bunt is primarily a problem of soil contamination, the most effective method of control is to grow resistant varieties. Thus far, this method has been highly successful throughout the dwarf-bunt-infested area. However, its continued success will depend largely upon whether there are distinct pathogenic entities which cause dwarf bunt. Determination of this fact has been delayed because the dwarf-bunt disease cannot readily be reproduced by the usual process of seed inoculation with chlamydospores.

Physiologic races of the bunt fungi usually differ in one or more of several characters, the most important being pathogenicity. The dwarf bunt race differs from other races in at least 8 characters, including pathogenicity (5, 6, 7, 15). The possibility that pathogenic races of the dwarf-bunt fungus exist is suggested by the fact that distinct entities based on other criteria are readily recognized. For example, among nine collections studied, distinct differences were noted in spore germination, spore size and markings, nuclear behavior, number of sporidia produced, and effect on host morphology.

In a preliminary report (5) on dwarf bunt, it was shown that the chlamydospores were nonviable under laboratory conditions, germination being limited to certain hyaline, apparently haploid spores. Later (6) it was found that germination of the regular diploid spores could be induced by prolonged soaking in water at a low temperature. It appeared that this information might facilitate further studies on the dwarf bunt, especially in studies where spore germination is essential, as in pathogenicity. Attempts were made, therefore, to determine the pathogenicity of dwarf-bunt collections from different areas of infestation in the Pacific Northwest. The spores were treated by presoaking in water in the refrigerator for six months. These spores were then used to inoculate the seed of the winter wheat host testers for common bunt races (11). Duplicate nurseries were grown at Pullman, Washington, and Bozeman, Montana. The results were negative, no smut being produced on any of the varieties, despite the fact that at least some germination of spores occurred in all of the collections under laboratory conditions. The incubation period required for germination in the laboratory, however, was 4 to 6 weeks. Assuming that a similar incubation period is required under field conditions no infection could

be expected, since wheat seedlings probably develop beyond the susceptible stage within that time.

Among the nine collections of dwarf bunt studied, spore germination ranged from a trace in a collection from Bozeman, Montana, to as high as 50 per cent in a collection from Troy, Idaho. The presoak treatment (6) was essential to germination of the spores of all collections except the one from Troy, Idaho. Spores of this collection, without presoaking, germinated up to 50 per cent after incubating 4 weeks. Presoaking the spores of this collection increased the speed of germination slightly but not the percentage of germination. In general, presoaking for periods longer than 6 months did not increase either speed or percentage of germination in any of the collections, and periods longer than 12 months were detrimental to germination. In the collection from Troy, Idaho, no germination occurred in spores that were presoaked 21 months while this same length of treatment did not greatly impair germination in a collection from Waterville,
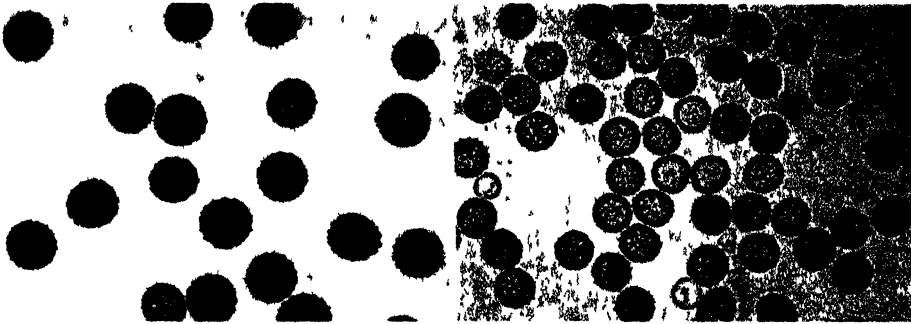
FIG. 1. Photomicrograph illustration of difference in size of chlamydospores of two collections of dwarf bunt. Left, collection from Bozeman, Montana; average diameter, about 20 microns. Right, collection from Troy, Idaho; average diameter, about 18 microns.

Washington. Thus, on the basis of difference in spore germinability there is evidence that dwarf bunt is caused by distinct physiologic entities of *Tilletia caries.*

Chlamydospore characteristics may also be used to differentiate between entities of the dwarf bunt. Prominence of the spore wall reticulations is one of the features which distinguish dwarf bunt from the common bunt races of *Tilletia caries.* However, there are differences in the degree of prominence of reticulations in various collections of dwarf bunt. For example, the spores of the collection from Waterville, Washington, are less prominently reticulate than those of the collection from Bozeman, Montana. Furthermore, the spores of the latter are larger and darker in color than those of the former collection. Spores of the collection from Bozeman are also larger than those of the collection from Troy, Idaho (Fig. 1).

Further indication of specialization in dwarf bunt is the difference in number of primary sporidia produced by the spores of collections from

different areas. There was also a difference in the tendency for promycelial branching. The number of sporidia on 11 germinating spores of the collection from Utah ranged from 28 to 66. Branching of the promycelia was common in this collection and the number of sporidia on the different branches varied. One promycelium had three branches with 13, 17, and 20, or a total of 50 sporidia. Only one spore had as many as 66 sporidia. The promycelium of this spore had two branches, one with 36 and the other with 30 sporidia. The average number of sporidia on these 11 promycelia was 45. The number of sporidia on the promycelia of 50 germinating spores of the collection from Troy, Idaho, ranged from 9 to 20, more than half of them ranging between 13 and 16. The average number of sporidia

FIG. 2. Photomicrograph showing the aggregation of nuclei into two groups in the promycelia of three germinating chlamydospores of one collection of dwarf bunt.

for these 50 promycelia was 14.8. Branching of the promycelia in this collection was the exception rather than the rule.

Nuclear behavior in different collections of dwarf bunt suggests specialization in this respect also. In general, the nuclear phenomena in dwarf bunt resemble those of common bunt. The promycelium is multinucleate and one nucleus passes into each primary sporidium. Fusion of the primary sporidia is followed by the migration of the nucleus from one sporidium into the other, thus initiating the dikaryophase. Secondary sporidia, which are the product of nonfused primary sporidia, usually are uninucleate but occasionally may be binucleate.

A notable feature of the nuclei in the promycelia of germinating dwarf bunt spores was their tendency to be aggregated into two groups, one

group near the base and the other near the tip (Fig. 2). In the collection from High Prairie, Washington, the nuclei were aggregated into two groups in 45 out of 51 promycelia observed. In the collection from Spangle, Washington, 140 out of 164 promycelia had the nuclei in two groups. In two other collections the nuclei were aggregated into two groups in less than half of the 52 promycelia observed. Although these observations suggest that dwarf-bunt collections may be specialized in this respect, there is need for further study of the nuclear behavior.

The reaction of the variety Orfed (C.I. 11913) to dwarf bunt at Troy, Idaho, and Bozeman, Montana, suggests specialization with respect to mor-
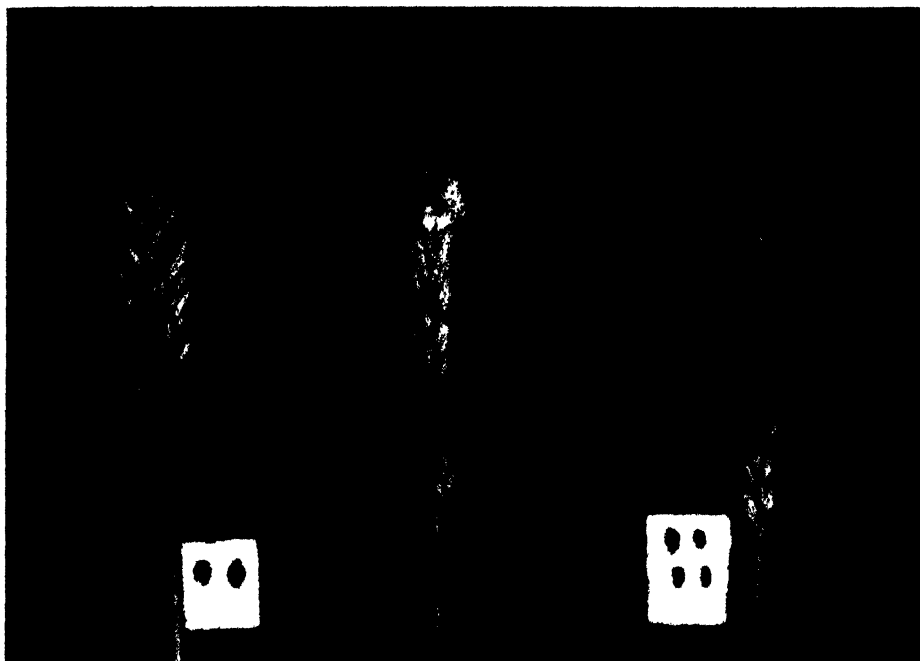
Fig. 3. Photograph showing difference in type of smutted head and smut balls produced by two collections of dwarf bunt on Orfed wheat. Center, healthy. Left, smutted head from Bozeman, Montana. Right, smutted head from Troy, Idaho.

phology of smutted heads and smut balls. Most of the smutted heads of this variety at the former location are slender and the smut balls are small, while at the latter location more vigorous smutted heads and smut balls are formed (Fig. 3). Thus, on the basis of this difference, together with the differences in chlamydospore morphology and rate of spore germination already mentioned, these two collections may be regarded as physiologic races.

It seems clear from the results presented here that there are specialized entities of *Tilletia caries* which cause dwarf bunt. These are not distinguished on the basis of pathogenicity and there is no evidence to indicate that pathogenic races do exist. However, since races do exist which are

distinguishable by other characters it seems probable that there also are pathogenic races.

Breeding for Dwarf-Bunt Resistance

Breeding for dwarf-bunt resistance in winter wheats has been a major phase of the wheat improvement program in the Pacific Northwest for many years. Relief (C.I. 10082), the first resistant variety to be developed, was distributed by the Utah Agricultural Experiment Station in 1934 (13). Four other dwarf-bunt-resistant varieties have been released since that time. These are listed in table 3. Three of the resistant varieties (Relief, Cache, and Wasatch) are hard red winter wheats. The other two, Rex and Hymar, are soft white wheats, representing the common and club types, respectively. These five varieties have a sufficiently broad geographic adaptation to cover the dwarf-bunt-infested Pacific Northwest.

TABLE 3.—*Dwarf-bunt-resistant varieties released for commercial production*

Variety	C.I. Number	Pedigree	Source of resistance	Releasing station
Relief	10082	Hussar × Turkey (Utah No. 26)[a]	Hussar	Utah
Cache	11599	Ridit × Turkey (Utah No. 26)	Ridit	Utah
Wasatch	11925	Relief × Ridit	Relief, Ridit	Utah
Rex	11689	White Odessa × Hard Federation	White Odessa	Oregon
Hymar	11605	Hybrid 128 × Martin	Martin	Washington

[a] Selection from Turkey, C.I. 2996, which has no known factors for resistance to bunt.

However, these are not perfect wheats and there is a continuous effort to develop better varieties and still maintain dwarf-bunt resistance.

Investigations and practical experience have demonstrated that dwarf bunt is most effectively controlled by the Martin-Hussar gene group (3, 8, 12). In addition, moderate resistance to dwarf bunt is found in Ridit but the protective degree of this resistance is not sufficient under severe epiphytotic conditions (9). For this reason the former type of resistance is more important than the latter, in breeding wheats with high resistance to dwarf bunt. The practical implications of this established fact are well illustrated in the results of wheat breeding in the Pacific Northwest in recent years (Table 4).

The first 24 hybrid selections listed in table 4 carry the Martin-Hussar type of resistance and are highly resistant to dwarf bunt. The next three carry the Ridit type of resistance and are moderately resistant to dwarf bunt. The last two selections listed in table 4, which carry only the Turkey (4) type of resistance, are highly susceptible to dwarf bunt. In the light of this information, therefore, the Martin gene appears to be the most important resistance factor in the development of dwarf-bunt-resistant varieties.

There is some evidence that Wasatch carries a combination of resistance factors that give a higher degree of resistance to dwarf bunt than does the Martin gene. Wasatch almost invariably has less dwarf bunt than do varieties with only the Martin type of resistance. In a test at Bozeman, Montana, in 1946 Wasatch had 0.5 per cent of dwarf bunt while Hussar (C.I. 4843), Martin (C.I. 4463), White Odessa (C.I. 4655), and Rex (C.I. 10065) had 2, 2.5, 3, 5, and 7 per cent, respectively. This apparent differ-

TABLE 4.—*The reaction to dwarf bunt of 29 hybrid selections of winter wheat, showing the relative value of three types of bunt resistance in breeding dwarf-bunt-resistant varieties*

Type of resistance, and hybrid	Selection number	C.I. number	State of origin	Reaction to dwarf bunt
Martin-Hussar Resistance				
Ridit × Relief	122a–327–1	11925	Utah	
Do	122a–70–3	11908	Do	
Do	112a–72–7	11909	Do	
Do	122a–20–3	11904	Do	
Do	122a–394–4	11905	Do	
Rex × Rio	381958	12234	Oregon	
Do	382165	12242	Do	
Do	381951	12246	Do	
Rex × Oro	382241	12421	Do	
Rio × Rex	382041	12422	Do	
Alicel × Rex	P-23		Do	Highly
Do	P-C16		Do	resistant
Do	P-C172		Do	
Do	P-80		Do	
Yogo × Wasatch			Montana	
Martin × Tenmarq		11804	Texas	
Do		11823	Do	
Do		11824	Do	
Turkey (C.I. 11530) × Oro[a]			California	
Brevon × Relief	109–45		Idaho	
Do	366–45		Do	
Brevon × Wasatch	754–45		Do	
Hussar × Kanred	105	11981	Nebraska	
Do	31B–96	11989	Do	
Ridit Resistance				
Brevon × Carlson's Fife	700–45		Idaho	Moderately
Ridit × Utah Kanred		11599	Utah	resistant
Composite Cross[b]	1–3–11–5	12385	Washington	
Turkey Resistance				
Alicel × Oro	P-10	12247	Oregon	Highly
Rio × Alicel	P-47	12377	Do	susceptible

[a] Thirteen of the selections from this cross that were tested were highly resistant to dwarf bunt.

[b] Brevon × (Oro × Turkey-Florence) (Oro × Fortyfold-Federation). Fifteen selections from this cross have been tested and found to be moderately resistant, thus reflecting at least the Brevon (Ridit) type of resistance.

ence, though only slight and of little practical significance, has been observed repeatedly under conditions of severe infestation. When infection percentages on susceptible varieties are relatively low (less than 50 per cent), this difference usually is not evident.

Plant breeders have been highly successful in the development of dwarf-bunt-resistant winter wheats. Some of the most promising selections are among those from Martin × Tenmarq, and Turkey (C.I. 11530) × Oro. If quality tests prove satisfactory, a new dwarf-bunt-resistant variety for Montana can be selected from this group. Most of these resistant lines, however, are not well suited to commercial production, and the problem remains of combining resistance to dwarf bunt with as many other desirable characteristics as possible.

Control Practices

Wherever dwarf bunt has occurred in destructive proportions one or more resistant varieties have been introduced to curb its onslaught. In Utah and certain parts of southern Idaho the introduction of Relief and Cache served to reduce the incidence of dwarf bunt from devastating proportions to almost negligible amounts. These varieties were largely replaced by the more resistant Wasatch. More recently, however, Cache has been increasing again because it yields slightly better and is more resistant to shattering than is Wasatch.

In the Gallatin and Flathead valleys of western Montana the dry-land winter-wheat industry was threatened with possible complete elimination by dwarf bunt in the period 1934–40. The introduction of Cache and Wasatch from Utah brought about a marked reduction in the amount of this disease within three years and, in fact, virtually saved the winter-wheat industry of that area.

The need for higher resistance than is carried by Ridit and Cache is well illustrated by the experience of introducing Cache into the Gallatin Valley of Montana. Under the severe conditions of infestation occurring in this area the highly susceptible variety Montana 36 had upwards of 75 per cent of dwarf bunt and Cache had only about 30 per cent in the most heavily infested fields. However, Wasatch under similar conditions had only a trace of dwarf bunt, thus demonstrating the superiority, under commercial conditions, of its resistance to this disease over that of Cache.

The most recent example of dwarf-bunt control through the introduction of a resistant variety is found in the infested area of Douglas County, Washington. Dwarf bunt is known to have been present in that area since 1940. It occurred in small amounts every year after that until 1945 when it reached severe proportions in at least two fields, one of Golden and the other of the Rio variety. Wasatch was introduced from Montana for seeding in 1946 and it proved to be satisfactory, both in field adaptation and in its resistance to dwarf bunt. Again the superiority of this type of resistance over the Ridit type was demonstrated. Adjacent plantings in the same field showed 10 per cent dwarf bunt in Ridit, as against traces in Wasatch. In 1948, when dwarf bunt reached destructive proportions in Rio and other susceptible varieties in this area, Wasatch was vir-

tually smut-free and in other respects appeared to be as good as or better than Rio, the predominant variety in that area.

SUMMARY

Dwarf bunt can seldom be reproduced artificially by seed inoculation. Repeated inoculations over a period of 15 years at experiment stations in the Pacific Northwest gave negative results in all cases except one.

Dwarf-bunt inoculum persists in the soil for two or more years, as shown by its occurrence in commercial wheat fields and by controlled experiments.

Dwarf bunt occurs in a wide range of climatic conditions and soil types. It is more prevalent and severe in dry-land areas but also attacks irrigated wheat. A compact soil favors the development of dwarf bunt.

The height of plants infected with dwarf bunt is only one-third to one-half that of noninfected plants, depending upon the variety. Among the varieties studied, the degree of dwarfing was greatest in Turkey and least in Triplet.

There is no evidence that pathogenically specialized races of dwarf bunt occur in the Pacific Northwest. Specialized entities based on chlamydospore germination, size of spores, prominence of spore wall reticulation, number of primary sporidia produced, branching of the promycelia, nuclear behavior, and host morphology were identified.

Only those varieties carrying the Martin-Hussar gene complex are highly resistant to dwarf bunt. Varieties with the Ridit type of bunt resistance are moderately resistant to dwarf bunt, while those with the Turkey factor (TT) are highly susceptible.

Plant breeders have been highly successful in the development of agronomically desirable wheats with resistance to dwarf bunt. Among commercial wheats in production in the Pacific Northwest, Relief, Wasatch, Cache, Hymar, and Rex are resistant to this disease. Many hybrid selections with dwarf bunt resistance are now available for breeding stock and some of these have value as potential new varieties.

Dwarf bunt can be controlled only by growing resistant varieties. This disease has been successfully controlled in Utah, southern Idaho, western Montana, and central Washington by the introduction of varieties with high resistance.

AGRICULTURAL EXPERIMENT STATIONS OF IDAHO, MONTANA,
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EFFECT OF FERTILIZATION ON THE RECOVERY OF TRANS-PLANTED SUGAR-BEET SEEDLINGS AFFECTED WITH APHANOMYCES COCHLIOIDES DRECHS. IN THE GREENHOUSE[1]

M. M. AFANASIEV AND H. E. MORRIS

INTRODUCTION

It is very important to have vigorous young beet seedlings prior to thinning in order to obtain a good stand. This is especially true when mechanical thinning of sugar beets is practiced. Very often a poor stand of sugar beets results because of seedling diseases or so-called "black root" of sugar beets.

Under Montana conditions when black root is severe, some beet seedlings die, although a large percentage of them recover. Damage to the crop, therefore, is primarily due to the retarded growth of plants of low vigor.

Extensive studies over a period of several years to determine the cause of seedling disease of sugar beets in Montana (1) showed that, although several different organisms may be involved in producing seedling disease of beets, *Aphanomyces cochlioides* is responsible for the major percentage of this disease in Montana.

In investigations relative to the occurrence and control of seedling diseases of sugar beets (2, 3, 4, 7) conducted in Montana, the effect of seed treatments, proper and balanced fertilization, preceding crops to sugar beets, temperature, and moisture were studied. It was found that a proper and balanced fertilization of the soil was the most important factor in the control of seedling diseases in Montana. Only small amounts of seedling diseases occurred in sugar beets planted in well fertilized soil, while large amounts occurred in soil low in the necessary nutrients.

Seed treatment was found to be only slightly beneficial in controlling seedling diseases of beets. The lowest amount of seedling disease occurred when beets were planted after corn; the highest, when beets followed beets. Diseases occurred in increasing amounts when beets were planted after corn, potatoes, oats, alfalfa, beans, and beets. Low temperatures and optimum moisture are more favorable to sugar beets from the standpoint of freedom from seedling diseases than are high temperatures and high moisture.

Since soil nutrients unquestionably have a great effect on the control of seedling diseases of sugar beets, it was decided to investigate the effects

of various nutrients and their combinations on the recovery of diseased beets and their subsequent yield under greenhouse conditions.

MATERIALS AND METHODS

Soil from the third-year alfalfa plot in Rotation 64 at the Huntley Branch Station, Huntley, Montana, was used in greenhouse flats holding about 24 lb. of soil. A high percentage of seedling diseases always oc-

TABLE 1.—*Effect of fertilization on recovery of transplanted diseased sugar-beet seedlings in the greenhouse, Bozeman, Montana, 1948*

Soil treatment	Degree of disease of transplanted seedlings	Data on harvested plants				Color of plants
		Number healthy	Av. height	Weight of single plant		
				Top	Root	
			cm.	gm.	gm.	
N	Healthy	6	9	6.3	0.5	dark green
	Slight	6	8	3.7	0.3	dark green
	Severe	6	9	6.2	0.5	dark green
P	Healthy	6	13	5.7	1.7	yellow green
	Slight	5	13	5.2	1.7	yellow green
	Severe	6	13	5.0	1.2	yellow green
K	Healthy	6	7	2.9	0.6	light green
	Slight	6	8	2.7	0.3	light green
	Severe	6	7	1.6	0.2	light green
M	Healthy	6	17	12.3	2.9	light green
	Slight	5	15	6.6	1.9	light green
	Severe	6	13	6.8	1.8	light green
NP	Healthy	6	25	41.7	5.2	green
	Slight	6	24	20.5	1.5	green
	Severe	6	25	28.0	2.2	green
PM	Healthy	6	16	9.7	3.3	light green
	Slight	5	14	7.4	2.6	light green
	Severe	6	14	8.0	2.3	light green
NPK	Healthy	6	34	43.0	3.5	green
	Slight	6	29	46.3	4.0	green
	Severe	4ᵃ	22	25.4	1.8	green
NPKM	Healthy	6	36	68.3	6.2	green
	Slight	6	23	20.5	1.0	green
	Severe	6	26	40.8	4.0	green
Check	Healthy	6	8	3.5	0.3	green
	Slight	6	6	2.2	0.3	green
	Severe	6	5	1.7	0.3	green

ᵃ One plant was diseased at harvest time.

curred in this type of soil in previous studies. Four rows of segmented sugar-beet seeds were planted in several greenhouse flats. After approximately 1 month of growth the beet seedlings were carefully dug, washed, and graded into healthy, slightly diseased, and severely diseased plants. The graded beet seedlings were planted in nine soil flats filled with the same soil, to which the following fertilizers were applied:

1. N   4. M   7. NPK
2. P   5. NP   8. NPKM
3. K   6. PM   9. Check

Nitrogen (N) was used in the form of sodium nitrate in the amount of 31.4 gm. per flat; phosphorus (P) in the form of treble superphosphate in the amount of 3.6 gm. per flat; potassium (K) in the form of potassium chloride in the amount of 9.4 gm. per flat; and manure (M) in the amount of $\frac{1}{5}$ of soil volume. Fertilizers were added in a dry form and thoroughly mixed with the soil.

Three rows of six sugar-beet seedlings each were planted in each flat of soil. One row was planted with healthy beet seedlings, the second with slightly diseased, and the last row with severely diseased seedlings. Water was added on the surface until the soil was uniformly moist.

Beets were grown in these flats approximately 2 months. At the end of this period the beets were dug and washed. Readings were taken on the number of healthy and diseased beet seedlings harvested. The height and weight of beets from each row was determined and their color was recorded. The results of these readings are presented in table 1.

RESULTS AND DISCUSSION

The results show that practically all diseased beets recovered. Similar results were obtained in Canada (6). Since the symptoms of diseased seedlings closely resembled those described for *Aphanomyces cochlioides* (1), it was assumed that this organism was mainly responsible for the disease. There was slight mortality in a few flats, and one beet remained diseased in a row planted with severely diseased seedlings in the soil fertilized with NPK.

The greatest difference in behavior of these beets was the amount of growth due to the different fertilizers added. The weight of the beets grown in soils fertilized with nitrogen, phosphorus, and potassium alone was slightly greater than the weight of those in the check. A more pronounced increase in weight occurred with the addition of manure and manure and phosphorus to the soil, and the greatest increase was with NP, NPK, and NPKM, especially the last (Table 1). Beets in the check remained very small. It has been shown that increasing the fertility of the soil, particularly with respect to phosphate, greatly reduces the losses due to *Aphanomyces cochlioides* Drechs. (5).

The tops of all transplanted seedlings grew more than the roots, which is natural since sugar beets develop leaves during the first part of their vegetative period and the root begins to increase in size later.

Healthy transplants, in practically all cases, produced the heaviest tops and roots. In some instances the rows planted with severely diseased seedlings produced plants heavier than those planted with slightly diseased seedlings. This was because of the crowding of the plants in the flats, with healthy and severely diseased seedlings in the outside rows and the slightly diseased seedlings in the middle rows.

The tops of the harvested beets varied from yellow-green to deep green in relation to the supply of nitrogen available to the plants.

Although the diseased sugar-beet seedlings showed a remarkable ability for recovery, this recovery was always delayed and hindered by the lack of available nutrients in the soil. It appears that if fertility of the soil is low and the beet seedlings have a considerable amount of disease, their condition may be improved by early application of nutrients.

This experiment was repeated twice with practically the same results. The result of only one experiment is reported.

SUMMARY

One-month-old diseased sugar-beet seedlings grown in the greenhouse and transplanted to soil flats fertilized with various combinations of nitrogen, phosphorus, potassium, and manure showed remarkable ability for recovery, especially in flats which received complete fertilization or manure. A row of healthy beet seedlings and two rows of diseased seedlings were planted in each flat of soil. Diseased seedlings grown in well-fertilized soil produced large plants. The seedlings grown in soil fertilized with nitrogen, phosphorus, and potassium alone, or grown in the check soil, remained very small.

These experiments show that if the fertility of the soil is low, and the beet seedlings have a considerable amount of disease, their condition may be improved by an early application of nutrients.

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FACTORS AFFECTING THE SURVIVAL OF HELMINTHO-
SPORIUM SATIVUM AND FUSARIUM LINI IN SOIL[1]

A. A. ANWAR

It has long been known that soil cropped continuously to flax is likely to become "wilt-sick" because *Fusarium lini* Bolley accumulates and persists in the soil. On the other hand, *Helminthosporium sativum* P., K., and B. does not seem to accumulate to so great an extent. The contrast between the behavior of the two organisms in the soil is very conspicuous in two contiguous plots at University Farm, St. Paul, Minnesota. One has been cropped continuously to flax since 1912, and the other has been cropped continuously to barley since 1920. Abundant inoculum has been added to these two plots periodically. The flax plot has become so thoroughly infested that 100 per cent of the plants of susceptible varieties always become infected and usually almost 100 per cent are killed. On the contrary, barley still grows well in the Helminthosporium plot despite attempts to attain thorough infestation of soil with *H. sativum*.

There is limited information regarding the survival of *Helminthosporium sativum* and *Fusarium lini* in soil. Bolley (3) and Bolley and Manns (4) reported that *F. lini* is the principal cause of soil sickness, and that it accumulates in the soil with successive cropping of flax. Sanford (11) and Henry (7) have stated that very little disease occurred on wheat or barley in the fields artificially inoculated with *H. sativum*.

Henry (6) found that the natural microflora of the soil had a marked inhibitive effect on the growth of *Helminthosporium sativum*. Sanford and Cormack (12) demonstrated that the addition of certain fungi, bacteria, and Actinomycetes to the soil reduced the destructive effects of *H. sativum*. No extensive work has so far been reported, however, on the relation of soil microflora to the development of flax wilt and the accumulation of *F. lini* in soil.

The writer therefore attempted to find out why *Fusarium lini* develops and persists in the soil, while *Helminthosporium sativum* does not. As the soil type of the two University Farm plots is the same and the weather conditions obviously are the same, and as the cropping history is so well known, the two plots offered exceptional opportunity for comparative studies.

MATERIALS AND METHODS

Four races each of *Helminthosporium sativum* and *Fusarium lini* were

isolated from diseased barley and flax plants, respectively, and were designated as races on the basis of differences in pathogenicity and cultural characters. These races[2] are referred to as races 1, 2, 3, and 4. A mixture of the four races of each pathogen was used throughout the investigation, unless otherwise indicated.

Three varieties of barley (Barbless C.I. 5105 (Wisconsin 38), Mars C.I. 7015, and Peatland C.I. 5267), and three varieties of flax (Crystal C.I. 982, Redwing C.I. 320, and Punjab C.I. 1115) were used.

Selected seeds of barley were treated in 1:1000 aqueous solution of New Improved Ceresan for 30 min. and then washed in running water and dried. The flax seeds were treated with New Improved Ceresan dust at the rate of 1.5 oz. per bu. of seeds. Only those lots which were found to have 95 to 100 per cent disease-free seeds in plating tests were used in the experiments.

Most of the greenhouse tests were made in a steamed soil comprising two parts loam and one part sand. The soil type of University Farm plots (barley plot and flax-wilt nursery) is Waukegan silty loam, with a pH about 5.7.

The effect of *Helminthosporium sativum* on barley was expressed as a disease index, which was computed after grouping the plants into the following five disease-severity classes: 1. Plants with no disease symptoms; severity, 0 per cent. 2. Plants with light brown lesions on the coleoptile; severity, trace to 30 per cent. 3. Plants with dark brown lesions on the coleoptile; severity, 31 to 60 per cent. 4. Plants severely stunted, crown and base of the plants partially decayed, and the root system partially destroyed; severity, 61 to 90 per cent. 5. Seedlings killed before or after emergence, root system, crown, and coleoptile completely destroyed; severity, 91 to 100 per cent.

The disease index was obtained by multiplying the number of plants in classes 1, 2, 3, 4, and 5 by 0, 15, 45, 75, and 95, respectively, summating all the resulting figures, and dividing by the total number of plants in all classes. The prevalence of *Fusarium lini* was determined by counting the number of plants wilted at the end of each experiment.

STUDY OF THE MICROFLORAL POPULATION OF THE SOIL

Isolations of fungi, bacteria, and Actinomycetes were made from the flax-wilt nursery and from the barley plot every month from June 1947 to May 1948, for the following purposes: 1. To determine the prevalence of *Helminthosporium sativum* and *Fusarium lini* in the barley plot and wilt nursery, respectively. 2. To obtain isolates of the most prevalent groups of fungi, bacteria, and Actinomycetes for the study of their effects on the growth of *H. sativum* and *F. lini*. 3. To study the seasonal variation in the

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[2] The designation of races as 1, 2, 3, and 4 is arbitrary, and these numbers are not the same as those used by other investigators in previous works.

microbial population of the soil in relation to the population of *H. sativum* and *F. lini*.

The plate-dilution method was used for the isolation of soil microorganisms. Dilutions of 1:1000 for the quantitative study of fungus flora and 1:100,000 for the determination of bacterial population of the soils were found to be the most suitable. Waksman's synthetic medium was used for fungi and albumin agar for bacteria.[3] Five random soil samples were taken from each plot, and 10 replicated plates were used for each sample and each dilution. Fungal and bacterial colonies in the respective plates were counted, and the number of fungi and bacteria per gm. of soil determined (Table 1). Isolates from all colonies of *Fusarium* that appeared

TABLE 1.—*The numbers of microorganisms per gram of soil in the flax-wilt plot and the barley plot at approximately monthly intervals from June 29, 1947, to May 30, 1948, inclusive*

Date of isolation	Microorganisms					
	In millions		In thousands			
	Bacteria and Actinomycetes		Fungi			
			Wilt plot		Barley plot	
	Wilt plot	Barley plot	Total	F. lini	Total	H. sativum
1947						
June 29	191	149	31	2.8	29	0.0
July 28	226	167	29	2.4	26	0.01
Aug. 14	158	157	27	2.9	31	0.0
Sept. 10	336	281	82	4.5	55	0.01
Oct. 13	300	230	88	5.1	56	0.01
Nov. 16	156	159	86	3.8	48	0.0
Dec. 14	32	17	20	1.6	14	0.0
1948						
Jan. 14	41	16	25	1.4	17	0.0
Feb. 15	19	21	19	1.4	21	0.0
Mar. 29	224	221	52	3.8	47	0.0
Apr. 25	190	181	42	2.3	36	0.01
May 30	132	182	43	2.5	22	0.0

in the dilution plates of the soil samples taken from the flax-wilt nursery were tested for their pathogenicity on Punjab flax, in order to distinguish *F. lini* from other soil Fusaria. The pathogenicity of *Helminthosporium sativum* obtained from the barley plot was tested on Barbless barley.

The population of *Fusarium lini* in the wilt nursery averaged about 3,000 spores and mycelial fragments per gm. of soil, whereas only 4 colonies of *Helminthosporium sativum* were obtained from the barley plot.

The data in table 1 show that the population of soil microorganisms

[3] Waksman's medium: agar, 20 gm.; dextrose, 10 gm.; peptone, 5 gm.; potassium dihydrogen phosphate, 1.0 gm.; magnesium sulphate, 0.5 gm.; water, 1000 ml. Adjust to pH 4.0.

Albumin agar: agar, 15 gm.; powdered egg albumin, 0.25 gm.; glucose, 1 gm.; magnesium sulphate, 0.20 gm.; potassium monohydrogen phosphate, 0.50 gm.; ferrous sulphate, trace; water, 1000 ml. Adjust to pH 7.0.

differed in different months of the year. The population of bacteria and fungi was highest in September–October and lowest in January–February. There seems to be a general rise in March and then a gradual decline from April to August, with an increase to the maximum in September and October. The population of *Helminthosporium sativum* was generally low and no important fluctuations were evident.

These data indicate that the conditions favoring growth and multiplication of other organisms also favored *Fusarium lini*. It is evident, however, that *Helminthosporium sativum* either does not grow and multiply appreciably in the soil, or else its presence is not detectable with the techniques used. The isolation of *H. sativum* was no more frequent when a number of differential media was used or soil was plated directly than when the soil dilution method was used.

THE EFFECT OF SOIL MICROORGANISMS ON THE GROWTH OF HELMINTHOSPORIUM SATIVUM AND FUSARIUM LINI IN CULTURE MEDIA

In these tests 86 isolates of fungi, bacteria, and Actinomycetes obtained from the barley plot and flax-wilt nursery at University Farm were tested to determine their effect on the growth of *Helminthosporium sativum* and *Fusarium lini* on 1.5 per cent potato-dextrose agar, which was the most suitable medium for the growth of most of the organisms tested. Three differ-
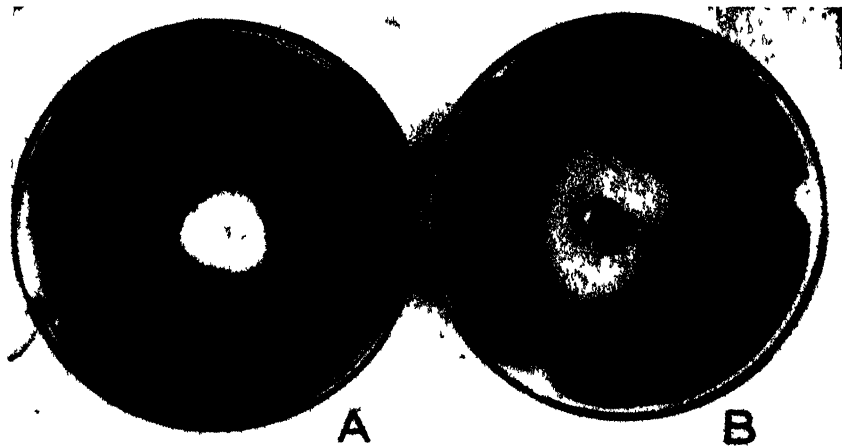
FIG. 1. A. Moderate antibiotic effect of *Penicillium* sp. on *Helminthosporium sativum* in culture. B. Strong antibiotic effect of *Bacillus subtilis* on *H. sativum* in culture.

ent methods of testing were used: 1. The test organism was streaked in the center of a Petri dish and *H. sativum* or *F. lini* placed on each side of it. 2. The center of the Petri plate was inoculated with the test organism, and *H. sativum* or *F. lini* placed around it. 3. Spore suspensions of *H. sativum* or *F. lini* were poured in Petri plates and the test organism was streaked through the center of the plate. All experiments were made in triplicate.

The effects observed were classified according to the zones of inhibition evident in the plates. In class 0, no inhibition was observed. Classes 1, 2, 3, and 4 were based on inhibition zones of 1 mm., 1 to 3 mm., 3 to 5 mm., and more than 5 mm., respectively. Class 4 also was characterized by malformed hyphae of *H. sativum* and *F. lini* bordering the inhibition zone. Classes 0 and 1 represent slight effects, classes 2 and 3 moderate effects, and class 4 a strong effect (Fig. 1). The degrees of antibiosis are summarized in table 2.

TABLE 2.—*The degree of antibiosis of different isolates of species of bacteria, Actinomycetes, and fungi, against Helminthosporium sativum and Fusarium lini on potato-dextrose agar*

Antibiotic organism	No. of isolates tested	No. of isolates in each antibiotic class[a]									
		H. sativum					F. lini				
		0	1	2	3	4	0	1	2	3	4
Bacillus subtilis	34	10	0	4	3	17	21	3	6	4	0
Actinomyces spp.	12	6	0	4	3	0	11	1	0	0	0
Penicillium luteum	34	1	0	2	1	0	4	0	0	0	0
P. oxalicum	2	1	0	0	1	0	1	0	1	0	0
P. notatum	1	0	0	1	0	0	1	0	0	0	0
P. rugulosum	1	0	0	1	0	0	1	0	0	0	0
Penicillium sp.	2	1	0	1	0	0	1	1	0	0	0
Aspergillus fumigatus	6	2	0	3	1	0	5	0	1	0	0
A. flavus	2	1	0	1	0	0	2	0	0	0	0
A. niger	1	0	0	1	0	0	1	0	0	0	0
Aspergillus spp.	4	4	0	0	0	0	4	0	0	0	0
Trichoderma lignorum	4	0	0	2	2	0	3	1	0	0	0
Fusarium spp.	3	2	0	0	1	0	3	0	0	0	0
Other genera	10	10	0	0	0	0	10	0	0	0	0
Total	86	38	0	20	11	17	68	6	8	4	0

[a] 0 = no effect; 1 = inhibition in a zone about 1 mm. wide; 2 = zone of inhibition 1 to 3 mm. wide; 3 = zone of inhibition 3 to 5 mm. wide; 4 = zone of inhibition 5 mm. or more with malformation of the hyphae bordering the inhibition zone.

Of 86 isolates tested, 34 were bacteria, 12 were Actinomycetes, and 40 were fungus isolates. The last group consisted of 10 isolates of *Penicillium*, 13 of *Aspergillus*, and 4 of *Trichoderma*, while 14 isolates belonged to other genera. Of the 34 isolates of soil bacteria, 24 were antibiotic to *Helminthosporium sativum*, 17 being strongly antibiotic (class 4) and 7 moderately so (classes 2 and 3). Ten bacterial isolates were moderately antibiotic to *Fusarium lini* and 3 inhibited the growth slightly (class 1). The isolates of bacteria that were antibiotic to *H. sativum* and *F. lini* were Gram-positive spore formers of the *Bacillus subtilis* Cohn emend. Prazmowski group. Only 7 of 12 isolates of Actinomycetes were moderately antibiotic (classes 2 and 3) to *H. sativum* and only one slightly antibiotic to *F. lini*.

Of 23 isolates of *Penicillium* and *Aspergillus* tested, 13 were moderately antibiotic to *Helminthosporium sativum* and 3 to *Fusarium lini*. One isolate of *Penicillium* sp. slightly inhibited the growth of *F. lini*.

The 4 isolates of *Trichoderma lignorum* (Pers. ex Fries) Bisby were all moderately antibiotic to *Helminthosporium sativum*, whereas only one of them inhibited the growth of *Fusarium lini* in culture, and its effect was slight. One isolate of *Fusarium* inhibited (class 3) the growth of *H. sativum*. Among 8 other genera of fungi tested, none was antibiotic to either *H. sativum* or *F. lini.*

Further studies with the metabolic products of certain strains of *Bacillus subtilis* and species of *Penicillium, Aspergillus,* and *Trichoderma* have shown that the bacterial filtrates conspicuously inhibited the growth of *Helminthosporium sativum* when 1 ml. of the filtrate was mixed with 20 cc. potato-dextrose agar. Filtrates of two strains of *Trichoderma lignorum* and one of *Penicillium oxalicum* Thom. also inhibited the growth of *H. sativum;* however, there was no marked inhibition in the growth of *Fusarium lini* on agar plates to which staling products of the antibiotic organisms were added.

The results suggest that a relatively large number of soil microorganisms may be responsible for repressing the population of *Helminthosporium sativum* in the soil, whereas *Fusarium lini,* which seems to be inhibited by relatively few, may be better adapted to grow and persist in the soil.

THE EFFECT OF ANTIBIOTIC ORGANISMS ON THE PATHOGENICITY OF HELMINTHOSPORIUM SATIVUM AND FUSARIUM LINI IN STEAMED SOIL

There is considerable evidence that the pathogenicity of soil-borne species of *Helminthosporium* can be materially reduced by the antibiotic effect of other soil-borne organisms. Greaney and Machacek (5) were able to control root rot of wheat, caused by *Helminthosporium sativum,* by inoculating steamed soil with *H. sativum* and *Cephalothecium roseum* Corda. Bisby, James, and Timonin (2) found that root rot of wheat caused by *H. sativum* was significantly reduced by the addition of *Trichoderma lignorum* to the soil.

The writer experimented to determine the relative effects of *Bacillus subtilis, Trichoderma lignorum,* and *Penicillium* sp. on the pathogenicity of *Helminthosporium sativum* and *Fusarium lini* in the greenhouse. The antibiotic tests were made in steamed soil in two series of pots, one for *H. sativum* and the second for *F. lini.* Each series comprised five sets, each set having four 6-in. pots. Four of these 5 sets were inoculated with the respective pathogens. The fifth set, to which steamed oat-wheat mixture was added, served as control. Inoculum of antibiotic organisms was added to 3 of the 4 sets infested with the pathogens. The antibiotic organisms used were *B. subtilis, T. lignorum,* and *Penicillium* sp., respectively.

The inoculum of the organisms was increased on oat-wheat mixture; about one pint of inoculum of the antibiotic organism and one pint of the pathogenic organism were mixed and added to four 6-in. pots.

TABLE 3.—*The disease index on Barbless barley and percentage of wilt on Punjab flax grown in steamed soil inoculated with Helminthosporium sativum and Fusarium lini, respectively, and with 3 antibiotic organisms*

Treatment	Disease index on barley		Treatment	Percentage of flax wilt	
	Exp. 1	*Exp. 2*		*Exp. 1*	*Exp. 2*
Control	0.4	0.7	Control	0	0
H. sativum + B. subtilis	0.7	0.9	F. lini + B. subtilis	88	80
H. sativum + T. lignorum	2.9	3.4	F. lini + T. lignorum	85	68
H. sativum + Penicillium	2.9	2.8	F. lini + Penicillium	87	77
H. sativum	4.2	4.0	F. lini	86	74

Analysis of variance of "disease index" data

Variance due to	DF	Sum of squares	Mean square	F	Significance	
					5 per cent	1 per cent
Replications	1	0.0689	0.0689	0.14	7.71	21.20
Treatments	4	19.25	4.81	29.19**	6.39	15.98
Error	4	0.1707				

** Highly significant.

The 20 pots of each series, which comprised 4 replicates and 5 treatments, were randomized on a greenhouse bench at about 80° F., and a new random arrangement was made every 4 days.

FIG. 2. Barbless barley grown at 80° F. in steamed soil infested with (A) no microorganisms, (B) *Helminthosporium sativum* and *Bacillus subtilis*, (C) *H. sativum* and *Penicillium* sp., (D) *H. sativum* and *Trichoderma lignorum*, (E) *H. sativum*.

The results are summarized in table 3. *Bacillus subtilis* was effective in decreasing the attack of *Helminthosporium sativum* and provided practical control against the seedling blight. Although *Trichoderma lignorum* and *Penicillium* sp. reduced the root rot of barley, they did not prevent it entirely. (See figure 2.) The analysis of variance of barley data show highly significant differences between treatments.

Although there was no control of flax wilt in pots to which antibiotic organisms were added, the wilting of flax seedlings started a week later in pots to which *Trichoderma lignorum* was added than in pots to which *Fusarium lini* alone was added.

FIELD EXPERIMENTS

Experiments in the field tested the influence of *Bacillus subtilis, Trichoderma lignorum,* and *Penicillium* sp. on the pathogenic effects of *Helminthosporium sativum* and *Fusarium lini*. There are no definite reports of the control of Helminthosporium root rot of barley and wheat by adding antibiotic organisms to normal soil. Sanford and Cormack (12) found that soil bacteria, Actinomycetes, and fungi reduced the pathogenic effects of *H. sativum* in different degree. Their results were based on tests made in steamed soil. According to Waksman (13), Chudiakov reported the control of flax wilt in Russia by adding an antibiotic soil bacterium to flax-sick soil. Beresova and Naoumova (1) stated that partial control of flax wilt could be attained by treating the flax seeds with *Pseudomonas* and *Achromobacter*.

TABLE 4.—*Blight and wilt development and yields in Barbless and Mars barley and Crystal and Redwing flax grown in field plots infested with Helminthosporium sativum and Fusarium lini, and with Bacillus subtilis, Trichoderma lignorum, and Penicillium sp.*

Crop and treatment	Disease development[a]		Yield (bu./acre)[b]	
	Barbless	Mars	Barbless	Mars
Barley				
Control	L −	L −	21.4	22.4
H. sativum + B. subtilis	L +	L	20.7	20.2
H. sativum + T. lignorum	M −	M	16.1	17.9
H. sativum + Penicillium sp.	M	M	17.3	18.7
H. sativum	H +	H +	12.6	12.1
	Crystal	Redwing	Crystal	Redwing
Flax				
Control	11	5	12.0	12.7
F. lini + B. subtilis	38	26	9.2	12.9
F. lini + T. lignorum	43	31	8.8	11.0
F. lini + Penicillium sp.	45	22	8.6	12.4
F. lini	47	24	8.7	11.0

[a] Infection in barley was light (L), moderate (M), or heavy (H), with plus and minus variation within each class. Infection on flax was determined as percentage of plants wilted.
[b] Least significant difference: for barley yields = 4.31; for flax yields = 1.80.

Fungal and bacterial inocula were grown as described previously and one pint of inoculum of a pathogen was mixed with one pint of inoculum of a test organism for distribution in an 18-ft. row. Where a pathogen was used alone, one pint of inoculum was mixed with one pint of sterilized oat-wheat mixture; and in the checks, 2 pints of sterile medium were added to the soil. The 5 treatments were randomized within each block, 3 replicates being made. The varieties used were Barbless and Mars barley and Crystal and Redwing flax. The severity of root rot of barley was determined at the end of the experiment by examining the plants in each row and grading the infection in each row as light, medium, or heavy. The severity of flax wilt was determined by counting the wilted plants in each row of each treatment. Data on yield served as additional evidence of the effect of different treatments. The data were analyzed statistically. The results are presented in table 4. The rows of barley to which *Helminthosporium sativum* and an antibiotic organism were added had much lighter infection and yielded significantly more than those inoculated with *H. sativum* alone.

There was little evidence of antibiosis in the flax plot, as the severity of flax wilt in rows treated with antibiotic organisms and in those inoculated with *Fusarium lini* alone was not conspicuously different. However, *F. lini* alone caused twice as much wilt on Crystal as on Redwing and yield of flax seemed to be related to the percentage of plants wilted.

Tests were also made to find the effect of soil moisture on the antibiotic activity of microorganisms against *Helminthosporium sativum* and *Fusa-*

TABLE 5.—*Blight and wilt development in Barbless and Mars barley and Punjab and Redwing flax, respectively; in moist and dry soil infested with Helminthosporium sativum and Fusarium lini, and with Bacillus subtilis, Trichoderma lignorum, and Penicillium sp.*

	Disease development[a]			
Crop and treatment	Moist soil		Dry soil	
	Barbless	Mars	Barbless	Mars
Barley				
Control	L −	L −	L −	L −
H. sativum + B. subtilis	M	M −	L	L +
H. sativum + T. lignorum	M	M −	M −	M −
H. sativum + Penicillium sp.	M +	M −	M −	M −
H. sativum	M −	M −	H −	H
	Punjab	Redwing	Punjab	Redwing
Flax				
Control	16	3	14	7
F. lini + B. subtilis	100	16	82	28
F. lini + T. lignorum	100	11	70	18
F. lini + Penicillium sp.	100	8	77	21
F. lini	100	10	88	25

[a] Infection in barley was light (L), moderate (M), or heavy (H), with plus and minus variations within each class. Infection on flax was determined as percentage of plants wilted.

rium lini in the field. The soil treatments and the strains of antibiotic organisms used were the same as in the previous experiment. The antibiotic organisms did not control the wilt of flax in dry or in moist soil (Table 5). The antibiotic effect of *Bacillus subtilis* against *Helminthosporium sativum* was somewhat better in comparatively dry soil than in moist soil.

EFFECT OF TEMPERATURE ON THE SURVIVAL OF HELMINTHOSPORIUM SATIVUM AND FUSARIUM LINI IN SOIL

There are several reports in literature on the effect of soil temperature on the pathogenicity of *Helminthosporium sativum* and *Fusarium lini.* Jones, Johnson, and Dickson (8) reported that the maximum infection of wheat seedlings by *H. sativum* occurred at 30° C., and the highest percentage of flax wilted at soil temperature of 24° to 28° C. Jones and Tisdale (9) give 24° to 28° C. as the optimum temperature for flax wilt and 38° C. as the maximum. Apparently the effect of soil temperature on the survival of these organisms has not been investigated.

In these tests attempt was made to determine the prevalence and survival of *Helminthosporium sativum* and *Fusarium lini* in steamed and nonsterile soils. Steamed and nonsteamed soil from the barley plot and the wilt nursery were infested with race 4 of *H. sativum* and race 4 of *F. lini*, respectively. Each soil lot and a water suspension of mycelial fragments and spores of the appropriate actively growing pathogen were mixed thoroughly before distribution to individual tins and glass jars. These containers were then stored in incubators at 8° to 10° C., 20° to 22° C., 35° to 37° C., and 45° to 50° C. One lot was kept at −15° to 0° C., the temperature range out of doors at University Farm, from December 29, 1948, to January 12, 1949. Control lots of soil received no inoculum.

Sterile water had been added to adjust the moisture content to 8 per cent of the dry weight of the soil. Weighing the containers twice daily and adding sterile tap water at incubator temperature to make up for the weight lost by evaporation maintained the moisture at the 8 per cent level. The storage time was recorded as soon as the soil temperature reached the temperature of the storage incubators.

Three replicate containers of infested soil and one of noninfested soil were removed from each incubator every 3 days for 15 days. They were placed in the greenhouse at about 80° F., and 10 seeds of Punjab flax or Barbless barley were planted in each. Three replicate cans of freshly steamed soil and 3 of natural soil were newly infested with the respective pathogens at each 3-day interval and were planted with flax or barley. These cans served as additional controls and provided data on the disease reactions of plants grown in newly infested soil which could be compared with data for stored soils. The prevalence and survival of *Helminthosporium*

sativum and *Fusarium lini* in these soils was judged by the disease index for the root rot of barley and by the percentage of flax wilt (Table 6).

Between −15° C. and +22° C. both *Helminthosporium sativum* and *Fusarium lini* survived storage better in steamed soil, where there were fewer biologic competitors than in the natural field soil.

Helminthosporium sativum survived storage at 35° to 37° C. but was almost eliminated within 6 days when storage temperature was as high as 45° to 50° C. *Fusarium lini*, on the other hand, was rapidly eliminated from infested steamed soil stored at 35° to 37° C., but survived fairly well in the infested natural soil stored at that temperature. Higher temperatures were detrimental to the survival of *F. lini*.

TABLE 6.—*The amount of disease that developed on Barbless barley and Punjab flax in steamed and natural soils previously inoculated with 4 races each of Helmintho- sporium sativum and Fusarium lini, and then kept 3 to 15 days at temperatures ranging from −15° to 50° C. prior to planting*

Storage		Disease index for barley		Percentage of flax wilt	
Temperature in degrees C.	Time (days)	Steamed soil	Natural soil	Steamed soil	Natural soil
−15 to 0	3	3.3	0.9	96	45
	6	2 1	2.3	50	37
	9	2.8	1.7	76	55
	12	2.2	1.4	73	23
	15	2.2	1.5	81	33
8 to 10	3	3.2	1.3	96	71
	6	3.6	1.3	100	50
	9	2.0	1.9	95	69
	12	2 4	1.1	95	69
	15	1.5	1.6	100	55
20 to 22	3	2.7	1.1	93	83
	6	3.7	1.9	96	60
	9	2.0	1.4	100	89
	12	2 3	1.0	90	79
	15	1.8	1.4	100	95
35 to 37	3	3.1	2.1	36	43
	6	3.7	0.9	8	22
	9	2.5	1 9	0	50
	12	1.6	0.9	0	13
	15	1.8	1.0	0	25
45 to 50	3	1.9	1.2	11	0
	6	0.9	0.9	0	5
	9	0.0	0.0	0	0
	12	0.0	0.0	0	0
	15	0.0	0.0	0	0
Controls: Newly infested soil, not stored	3	3.9	1.6	96	91
	6	2.9	2.1	100	75
	9	3.9	1.9	95	84
	12	3.9	2.1	95	87
	15	2.8	1.8	100	100

Differences in the survival of *Helminthosporium sativum* and *Fusarium lini* are not very striking. Nevertheless, at higher temperatures *H. sativum* seems to have an advantage over *F. lini*. It is unlikely, however, that either summer or winter soil temperatures in Minnesota would be limiting factors in the survival of the two pathogens.

RELATION OF STUBBLE TO THE SURVIVAL OF HELMINTHOSPORIUM SATIVUM AND FUSARIUM LINI IN SOIL

Infested plant stubble was gathered from the barley plot and the wilt nursery and added to steamed soil in 1 : 10, 2 : 10, and 4 : 10 ratios. In another series, soil was taken from the barley plot and the wilt nursery, cleaned of all stubble, and added to steamed soil in the same ratios. Three sets of pots were used as controls. The first set consisted of steamed soil, the second set was steamed soil plus inoculum of *Helminthosporium sativum* or *Fusarium lini*, and the third set was steamed soil plus steamed flax or barley stubble.

Four replicates were used for each treatment. Twenty-five disease-free seeds of Barbless barley or Punjab flax were planted in each plot. Two weeks after the emergence of plants the percentage of flax wilt was recorded. The barley plants were dug after two weeks, washed, and classified for the determination of disease index.

Root rot of barley was heavier in pots to which stubble had been added than in those to which infested soil was added. The disease indices of barley plants in pots with 1 : 10, 2 : 10, and 4 : 10 ratios of stubble to soil were 2.2, 1.5, and 3.0, respectively. In a similar soil series without stubble but with similar ratios of infested soil to steamed soil, the disease indices of plants were 0.7, 0.7, and 0.9, respectively. This definitely indicates that *Helminthosporium sativum* survives better on stubble than in soil.

These results substantiate those of Padwick (10), who showed that Helminthosporium root rot of wheat was considerably increased when infested root systems of grasses were added to soil.

The differences in the percentage of flax wilt were also conspicuous, although in exactly the opposite direction. The percentages of flax wilt in stubble series for the three ratios of 1 : 10, 2 : 10, and 4 : 10 were 12, 19, and 25, respectively; whereas in the soil series the percentages were 44, 59, and 69, respectively. There was 30 to 40 per cent more wilt in steamed soil to which infested soil was added than in soil to which infested stubble was added. This indicates that *Fusarium lini* may survive better in soil than on plant refuse.

DISCUSSION

Fusarium lini accumulates in soils continuously cropped to flax, whereas *Helminthosporium sativum* does not seem to accumulate to the same extent in soils cropped to barley and wheat.

In the present studies it was found that the difference in the sensitivity

of *Helminthosporium sativum* and *Fusarium lini* to antibiotic organisms of the soil is one of the important factors accounting for the fact that *H. sativum* is not able to accumulate and infest the soil to the same extent as *F. lini*.

In spite of the fact that large amounts of inoculum of *Helminthosporium sativum* have been added to the barley plot every year for the last 18 years, only 4 colonies of *Helminthosporium sativum* were obtained from the barley plot in plating-out studies with soil dilutions; whereas there were 3000 colonies of *Fusarium lini* per gm. of soil from the flax-wilt plot to which inoculum of *F. lini* was occasionally added. On the other hand, the total number of colonies of all organisms per plate was approximately the same for the two plots. Having established the fact that *F. lini* is very abundant in the soil of the flax plot while *H. sativum* is very rare in the barley plot, the question naturally arose as to the reasons for these differences. It was found that *F. lini* is relatively unaffected by other microorganisms in the soil while *H. sativum* is deleteriously affected by many.

Of 86 isolates of bacteria, fungi, and Actinomycetes, 48 were antibiotic to *Helminthosporium sativum*, whereas only 12 were decidedly antibiotic to *Fusarium lini* and none of them was strongly antibiotic. Of the organisms that were antibiotic to *H. sativum*, several isolates of *Bacillus subtilis*, *Penicillium* spp., *Aspergillus* spp., and *Trichoderma lignorum* were most effective, as measured both by the width of the zone of inhibition and the final effect on the colonies.

Not only was the antibiosis of certain organisms effective on agar media but it was evident that some of the organisms could protect plants against infection in the field and greenhouse. When steamed soil was infested with *Helminthosporium sativum* and planted with barley, the plants were severely and uniformly diseased and stunted and yield was reduced significantly. When the soil was infested with a mixture of *H. sativum* and certain isolates of *Bacillus subtilis*, on the other hand, the barley plants grew almost as well as plants in noninfested soil and yield was not lowered significantly. *B. subtilis* therefore gave almost complete protection against the attack of *H. sativum*. Partial protection resulted also from the use of *Penicillium* species and *Trichoderma lignorum*, but the results were not so striking as those with *B. subtilis*.

Similar greenhouse and field experiments with flax wilt gave no significant differences in the amount of wilt regardless of the incorporation in the soil of organisms that had protected barley from blight as well as those that had been antibiotic to *Fusarium lini* in culture.

Soil moisture may affect the antibiotic activity of soil microorganisms. The antibiotic effect of *Bacillus subtilis* is operative against *Helminthosporium sativum* in relatively dry soil.

Both *Helminthosporium sativum* and *Fusarium lini* survive in the soil over a rather wide temperature range. Both organisms are almost elimi-

nated if soil temperature rises to 45° and 50° C. for 3 days, but they do seem to survive temperatures as low as −15° C. for 15 days. Although the differences in the survival ability of the two organisms at these soil temperatures is very slight, *H. sativum* tolerates high temperature somewhat better than does *F. lini*. It seems improbable that temperatures as high as those used in these experiments would prevail often enough or long enough under natural conditions to account for the disappearance of either organism from the soil. What little advantage there is, however, seems to be with *H. sativum*. Consequently, these facts increase the probability that the effect of antibiotic organisms on *H. sativum* and their relative ineffectiveness on *F. lini* account to a considerable extent for the difference in survival ability in soil.

The limited survival of *Helminthosporium sativum* in the soil seems to be due to its ability to persist for some time on stubble, and it probably does survive on stubble that has been plowed under until antibiotic organisms reduce its ability to grow and multiply profusely in the soil.

Although factors other than antibiosis may influence the survival of *Fusarium lini* and *Helminthosporium sativum* in the soil, the conclusions seem justified that under the experimental conditions and in the soil type used by the writer, the difference in the sensitivity of the two organisms to antibiotic microflora in the soil is a major factor in the survival of the two organisms.

SUMMARY

The prevalence of *Fusarium lini* in the flax-wilt nursery to which inoculum of *F. lini* was occasionally added was 3000 spores or mycelial fragments per gm. of soil. On the other hand, the prevalence of *Helminthosporium sativum* in the barley plot, to which inoculum of *H. sativum* was added annually, was not more than 10 per gm. of soil and frequently this fungus could not be recovered from the plot. There seems to be a close association between the high counts of *F. lini* in the wilt nursery and the high incidence of flax wilt, and between the infrequent isolation of *H. sativum* and the comparatively low incidence of root rot.

The population of soil bacteria, soil fungi, and *Fusarium lini* varied with seasonal changes, being highest in September–October and lowest in January–February. The population of *F. lini* followed the same trend as that of other fungi and bacteria. This fact indicates that the conditions optimum for the growth of other soil microorganisms are optimum for *F. lini*.

Of a total of 86 isolates of bacteria, Actinomycetes, and fungi, 48 were antibiotic to *Helminthosporium sativum* and 12 to *Fusarium lini* when grown on potato-dextrose agar. The metabolic products of some of the bacteria, Actinomycetes, and fungi inhibited the growth of *H. sativum* when added to agar medium. The growth of *F. lini* was not conspicuously affected by the same products.

A soil isolate of *Bacillus subtilis*, when added to steamed soil with

Helminthosporium sativum, completely protected barley seedlings against root-rot. The *B. subtilis* also protected field plots of barley from heavy infection by the *Helminthosporium.* *Trichoderma lignorum* and *Penicillium* spp. also gave partial protection from the disease. Similar treatments failed to protect flax from wilt.

Barley grown in rod rows infested with mixtures of *Helminthosporium sativum* and one of the three antiobiotic microorganisms outyielded barley in the rows infested with *H. sativum* alone.

The antibiotic effect of *Bacillus subtilis* was operative against *Helminthosporium sativum* in relatively dry soil. The flax wilt was not controlled by the antibiotic organisms in either dry or moist soils.

Helminthosporium sativum survived temperatures of 45° to 50° C. for 6 days, both in steamed and natural soils. *Fusarium lini* was less able to survive high temperatures.

Addition of infested barley stubble to the soil increased the incidence of root rot more than did addition of soil infested with *Helminthosporium*. Addition of infested flax stubble to the soil increased the incidence of flax wilt but was less effective than the addition of soil infested with *Fusarium lini*.

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THE PATHOGENICITY OF SPECIES OF HELMINTHOSPORIUM ON CORN[1]

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Diseases of corn caused by *Helminthosporium turcicum* Pass. (4, 7) and *H. maydis* N. and M. (5) have long been known. Recently another species, *H. carbonum* Ullstrup, has become prevalent and destructive in the corn belt of the United States (11). Although species of *Helminthosporium* have been reported on corn in Minnesota, their relative prevalence and importance on different lines and varieties of corn and other gramineous hosts have not heretofore been studied. This paper presents preliminary results of work on this problem.

SOURCE AND IDENTITY OF HELMINTHOSPORIA

Helminthosporium carbonum, H. sativum, H. turcicum, and a long-spored isolate of *Helminthosporium*, designated in this paper as *Helminthosporium* Z[2], were isolated repeatedly from lesions on the leaves of corn obtained from several localities in Minnesota. Of the species isolated, *H. sativum* P., K., and B. was by far the most prevalent. Certain other fungi were frequently isolated, but were discarded as they were not pathogenic on corn. For a comparative study, several races of *H. sativum* from other sources were included in these tests.

Since *Helminthosporium* Z resembled *H. carbonum*, a detailed morphological study was made of monosporous isolates of the two. The spores used for comparison were produced on autoclaved wheat heads kept at room temperature (20° to 24° C.). Two hundred conidia of each isolate were measured 15 days after inoculation.

The conidia of *Helminthosporium* Z averaged 115 μ in length and 19 μ in width and the average number of septa was 9. They were from 36 to 238 μ long, although only a few spores longer than 200 μ were found. The width ranged from 10 to 26 μ, and the number of septa from 1 to 14. Typical conidia of *Helminthosporium* Z were usually slightly curved, elongate, and tapered slightly toward the rounded end. Conidia were dark olivaceous brown, and one or both ends might be subhyaline. Spores germinated in distilled water by the production of two polar germ tubes.

Conidia of *Helminthosporium carbonum* race 1 averaged 87 μ long and 21 μ wide, and had 8 septa. The difference in mean length of conidia of the

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[2] It seems desirable to designate the organism as *Helminthosporium* Z until there has been ample opportunity to study the range of variation within the species *H. carbonum*, as the organism may be an undescribed species of *Helminthosporium* or simply a variety or race of *H. carbonum*.

two Helminthosporium isolates was 25.80 ± μ; in mean width, 2.52 ± μ; and in the mean of the number of conidial septations, 0.99.

The symptoms produced by *Helminthosporium* Z resemble most closely those produced by races 1 and 5 of *H. sativum*[3]. Numerous small lesions, usually about 2 mm. in diameter and noncoalescent, are produced. The lesions are elliptical or oval, whitish to brownish, with a light-brown to brown border. The symptoms produced by *Helminthosporium* Z that are distinct from those produced by the other species of *Helminthosporium* are the chlorotic areas around the spots, and leaf curling and subsequent drying. Infected areas on the stems at first appear watersoaked but later become brownish. The organism also can cause rotting of the mesocotyl and roots. In this respect it behaves like *H. carbonum*.

The symptoms caused by *Helminthosporium sativum* on various cereals and grasses other than corn have been described by several workers (3, 4, 8,
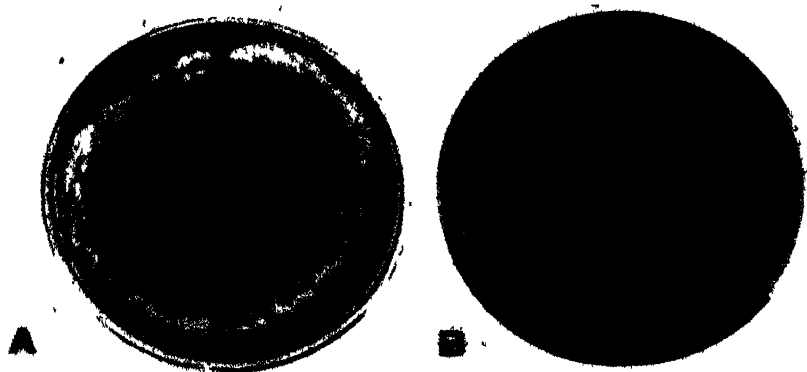
Fig. 1. *Helminthosporium* Z on two media: (A) potato-dextrose corn steep agar, and (B) potato-dextrose-cornmeal agar.

10). In general, the most typical symptoms produced by *H. sativum* on corn resemble those mentioned for *Helminthosporium* Z. Races 4 and 5, however, differ from the other races of *H. sativum* and from *Helminthosporium* Z in the size of lesions produced on the leaf sheath. Fully developed lesions produced by races 4 and 5 of *H. sativum* are irregular to oval and colorless, transparent straw color, or chocolate brown with a brownish to purplish margin. The largest lesions were 12 mm. long and 3 mm. wide and they seldom coalesced. Symptoms produced on seedling leaves as well as the symptoms of root rot agree with those reported for *H. sativum* on other cereals and grasses (3, 10).

Helminthosporium Z is extremely variable in cultural characters when grown on different nutrient media or under different environmental conditions (Fig. 1). Thus, *Helminthosporium* Z on one medium may resemble a race of *H. carbonum*, but may differ greatly on another.

[3] The numbers used in designating races of *Helminthosporium spp.* are not necessarily the same as those used by previous investigators.

PATHOGENICITY STUDIES

Relative Virulence of Species of Helminthosporium on Varieties of Corn in the Greenhouse

Preliminary pathogenicity tests were made with 26 cultural races of *Helminthosporium* on corn. From these tests, thirteen races were selected for more detailed study. Seed of five varieties of corn previously treated with Spergon were planted in autoclaved soil in 6-in. pots in the greenhouse. The inoculum was increased by growing the races for 20 days on 2 per cent potato-dextrose agar. Aqueous suspensions of inoculum were made by washing the cultures with sterile distilled water. This suspension was then strained through sterile cheesecloth and used to inoculate 30-day-old plants. Two methods of inoculation were tried: (a) injecting the inoculum into the second lowest internode with a hypodermic syringe, and (b) spraying the inoculum on the leaves with a small hand sprayer. After inoculation, the plants were kept for about 24 hr. in a muslin-covered moist chamber at approximately 27° C.

TABLE 1.—*Reactions of four varieties of corn to 13 races of Helminthosporium spp. when inoculated by injection and by spraying with conidial and mycelial suspensions in the greenhouse*

Race		Method of inoculation	Sweet corn[a]	Minhybrid 800	Minhybrid 500	Double cross D4
H. carbonum race 1		Injection	S	S	S	S
		Spray	S	S	S	S
Do	1–1[b]	Injection	MR	MR	MR	R
		Spray	MR	R	R	R
H. sativum race 1		Injection	MS	MS	MS	MR
		Spray	MR	MR	MR	MR
Do	2	Injection	MR	MR	MR	MR
		Spray	R	MR	MR	R
Do	3	Injection	MR	MR	MR	R
		Spray	R	R	R	R
Do	4	Injection	MS	MR	MR	R
		Spray	MR	MR	R	R
Do	5	Injection	MR	R	MR	R
		Spray	MR	MR	MR	MR
Do	6	Injection	R	MR	MR	R
		Spray	R	T	T	T
Do	7	Injection	MS	MS	MS	MS
		Spray	MR	MR	MR	R
Do	8	Injection	MR	R	R	R
		Spray	R	R	T	T
H. turcicum race 1		Injection	S	S	S	S
		Spray	S	S	S	S
Do	2	Injection	S	S	S	S
		Spray	S	S	S	S
Helminthosporium Z		Injection	S	MS	MS	MR
		Spray	MS	MS	MS	R

[a] S = susceptible; R = resistant; MR = moderately resistant; MS = moderately susceptible; T = trace.

[b] A variant of *H. carbonum* 1.

The plants were usually more severely infected when inoculated with a hypodermic syringe than when sprayed (Table 1 and Fig. 2.). In other experiments it appeared that when the inoculum was confined to the upper whorl of the plant, the infections were more or less alike irrespective of the method used.

Races 1 and 2 of *Helminthosporium turcicum* and race 1 of *H. carbonum* attacked all the varieties of corn about equally well. *Helminthosporium Z* infected all the varieties tested but the severity of attack was low on sweet

FIG. 2. Seedlings of sweet corn inoculated by means of hypodermic syringe with (A) *Helminthosporium sativum* race 7, (B) *Helminthosporium Z*, (C) *H. sativum* race 2, and check.

corn and on double cross D4 when the inoculum was sprayed on the leaves. There also were marked differences in pathogenicity among the races of *H. sativum:* Races 1, 2, 3, 4, 5, and 7 were moderately pathogenic on at least three varieties of corn, whereas races 6 and 8 were weakly pathogenic. A cultural variant of *H. carbonum* designated as *H. carbonum* race 1-1 was much less pathogenic than its parent, *H. carbonum* race 1. Therefore it also is a distinct parasitic race.

Reactions of South American Corn Selections to Two Races of Helminthosporium turcicum

Although races 1 and 2 of *Helminthosporium turcicum* were alike in pathogenicity in previous experiments, it was thought that some difference in virulence might be apparent on certain other varieties. Moreover, the reactions of South American corn selections to *H. turcicum* may be of practical importance. Ten South American selections were inoculated hypodermically with both races of *H. turcicum* Sweet corn was included as a

susceptible variety and noninoculated plants of each selection were used as controls.

Selections 10, 15, and 17 of South American corn were resistant, moderately resistant, and moderately susceptible, respectively, to *H. turcicum*

TABLE 2.—*Reactions of ten selections of South American corn to two races of Helminthosporium turcicum*

Selection No. of corn	Source[a] of selection	Races and host reaction		
		Race 1	Race 2	Control
3	Brazil	S	S	O
4	Do	S	S	O
7	Do	S	S	O
15	Do	S	MR	O
17	Do	S	MS	O
10	Brazil and Peru	S	R	O
12	Do	S	S	O
14	Do	S	S	O
8	Chile	S	S	O
16	Do	S	S	O
Sweet corn	Minnesota	S	S	O

[a] The seed corn from South America was furnished by Mr. George Hafstad of the Rubber Development Corporation, Amazon Division, Brazil.

race 2, while race 1 attacked all selections equally well (Table 2). It is noteworthy that race 1 attacked all the varieties of corn tested whereas race 2 did not. Evidently, therefore, races 1 and 2 of *H. turcicum* can be considered as distinct parasitic races.

Inoculations on Miscellaneous Gramineae

The host range of those species of *Helminthosporium* that attack corn is imperfectly known, but this information may be important in control practices. To obtain more definite information on this subject, the writer inoculated a number of crop plants with four species of *Helminthosporium* known to be parasitic on corn.

Helminthosporium turcicum has been reported on various wild and cultivated species of the Gramineae (1, 2, 4, 6, 7, 9) as well as on corn. At present *H. carbonum* has been reported only from corn and nothing is known about the host range of *Helminthosporium Z. H. sativum* is known to attack the common small grains and many wild grasses (3, 10). Extensive cross inoculations were made with 13 races of *Helminthosporium* spp. Clean and surface-disinfected seeds of 6 species of grasses were planted in 4-in. pots filled with autoclaved soil and placed in the greenhouse at about 26° C. The seeds were submerged for 10 min. in a 1:1000 solution of bichloride of mercury mixed with a wetting agent (Nacconol), then rinsed in a 0.5–1.0 per cent solution of chlorinated lime.

The 15-day-old seedlings of the various cereals and grasses were sprayed with conidial suspensions of the various races and incubated in a moist chamber for about 24 hr. The substrate used for increasing the inoculum

was a half-and-half mixture of autoclaved wheat and oat seeds. The results, summarized in table 3, show that the host range varies considerably with the species and race of *Helminthosporium*. *H. turcicum* and *H. carbonum* infected three of the Gramineae. Four of the races of *H. sativum* infected

TABLE 3.—*Reactions of 6 species of Gramineae to 13 races of Helminthosporium spp.*

Species and race	Host and reaction[a]					
	Marquis wheat	Bonda oats	Barbless barley	Golden Bantam corn	Teosinte	Sudan grass
H. carbonum race 1	O	O	O	S	S	—
Do　　　1–1	—	—	—	MR	O	T
H. sativum race 1	MR	O	R	MR	O	MS
Do　　2	O	O	R	R	O	MR
Do　　3	MS	O	MS	R	R	S
Do　　4	MS	O	S	MR	R	S
Do　　5	S	T	T	MR	R	S
Do　　6	MR	O	R	R	O	MS
Do　　7·	T	O	MS	MR	MR	MR
Do　　8	O	O	O	R	O	T
H. turcicum race 1	O	O	R	S	S	S
Do　·　2	O	O	O	S	MS	S
Helminthosporium Z	O	O	O	MS	R	S

[a] S = susceptible; MS = moderately susceptible; MR = moderately resistant; R = resistant; T = trace; O = immune; — = no test.

at least five of the six hosts, whereas race 2 attacked barley and corn weakly and was moderately pathogenic on Sudan grass.

Helminthosporium Z was only weakly pathogenic on teosinte, while race 1 of *H. carbonum* infected it severely. However, the variant 1–1 of this species did not attack teosinte. All the species of *Helminthosporium* attacked Sudan grass; none of them attacked oats except race 5 of *H. sativum*, which attacked oats weakly.

Field Experiments

The pathogenicity of several species of *Helminthosporium* was determined on 8 varieties of corn and one of teosinte in the field. Duplicate tests were made in July, 1946. Each plot consisted of 9 rows, 130 ft. long and 3 ft. apart. Eight varieties of corn and one variety of teosinte were planted. Two seeds were sown per foot and each plot was divided into 13 blocks, 12 of which were inoculated with a race of *Helminthosporium* by injecting a conidial suspension into the spiral of the corn plant shortly before tasseling. Check plants in the 13th block were injected with sterile broth. The organisms were increased on an autoclaved half-and-half mixture of wheat and oats. At the time of inoculation the plants were from 37 to 41 days old and from 2 to 4 ft. in height, depending on the variety and location of plot. Results are given in table 4.[4]

[4] The seed corn was furnished by the Division of Agronomy and Plant Genetics, University of Minnesota, and seed of teosinte by Dr. J. G. Harrar of the Rockefeller Foundation, Mexico.

Although the weather was cold and dry after inoculation, the results agreed well with those in the greenhouse except that *Helminthosporium turcicum* race 1 appeared much less virulent in the field than in the greenhouse. This apparent loss in pathogenicity may be due to mutation, as there had been obvious changes in cultural characters on artificial media.

There were not only clear-cut differences in the virulence of the various

TABLE 4.—*Field reactions of eight varieties of corn and one of teosinte to 12 races of Helminthosporium spp. when inoculated by the hypodermic method in the field*

	Host[a] and reaction[b]								
Species and race	Double crosses							Golden Bantam	Teosinte
	408	500	505	602	800	W240	D4		
H. carbonum race 1	S	S	S	S	S	MS	S	S	S
H. sativum race 1	MS	MS	MS	MS	MS	MR	R	MS	R
Do 2	MR	MS	MR	R	MR	MR	MR	MR	R
Do 3	R	MR	MR	MS	MR	R	MR	MR	MR
Do 4	S	MS	S	S	MS	MR	MS	MS	MS
Do 5	S	MS	MR	MS	MR	R	MR	MR	R
Do 6	MS	MS	MS	MS	MR	MR	MR	R	MR
Do 7	S	S	S	MR	MS	MR	MS	MS	MS
Do 8	MS	MR	MS	MR	MR	R	R	MR	R
H. turcicum race 1	MS	S	S	MS	S	S	S	S	S
Do 2	MS	MS	MS	MS	MR	R	S	MR	MS
Helminthosporium Z	S	MR	S	MS	MS	MS	S	S	R

[a] Varieties 408, 500, 505, 602, and 800 are Minhybrids; variety W240 is a Wisconsin hybrid; variety D4 is a Northrup, King and Co. hybrid.
[b] S = susceptible; MS = moderately susceptible; MR = moderately resistant; R = resistant.

races, but also differences in the resistance of corn varieties and teosinte. *Helminthosporium* Z and races 4 and 7 of *H. sativum* were almost as highly pathogenic as the virulent races of *H. carbonum* and *H. turcicum;* and races 1 and 5 of *H. sativum* were as virulent as *H. turcicum* race 1. Although teosinte plants were resistant to *Helminthosporium* Z, they were severely infected by *H. carbonum* race 1, which appears to be most closely related to *Helminthosporium* Z in many characters. These experiments prove that certain races of *H. sativum* and *Helminthosporium* Z may cause severe infection on corn in the field.

CONCLUSIONS

Helminthosporium turcicum, H. sativum, H. carbonum, and *Helminthosporium* Z cause diseases of corn in Minnesota. These Helminthosporia differed greatly in their virulence on 8 varieties of corn and one variety of teosinte. Races of *H. sativum* also differed greatly in pathogenicity and to some extent in symptoms produced on the foliage of corn. The identification of the casual agent by the symptoms alone would sometimes be difficult, because two or more Helminthosporia may attack the same leaf. In addition,

certain saprophytic fungi, at least under certain conditions, modify the type of lesion produced. Moreover, the symptoms vary to some extent with the variety of corn, the age of the plants, and the environmental conditions.

A large-spored *Helminthosporium* was isolated 'from diseased foliage of a corn plant. This organism was designated *Helminthosporium* Z until there was an opportunity to determine whether or not it was an undescribed species of *Helminthosporium* or a distinct variety or race of *H. carbonum*. *Helminthosporium* Z caused not only severe infection on the foliage of corn, but also stalk rot and root rot. In morphology it resembles somewhat *H. carbonum*, whereas the symptoms produced resemble more closely those produced by *H. sativum*. Although *Helminthosporium* Z may be a virulent pathogen on many varieties of corn, how prevalent and important it is in nature is not yet known.

Helminthosporium sativum is a virulent parasite on corn and teosinte. Although it has been known for a long time that this species comprises many parasitic races and that it has a very wide host range, it was not known that it was a common pathogen on corn.

The present studies have clearly shown that Helminthosporium diseases of corn constitute a complex problem and that there is need for much more work on the disease complex caused by *Helminthosporium* spp.

SUMMARY

Helminthosporium sativum was the most common pathogen isolated from diseased corn leaves in Minnesota. *H. carbonum*, *H. turcicum*, and *Helminthosporium* Z also were isolated and studied in considerable detail.

Helminthosporium sativum, not heretofore reported as pathogenic on corn, attacks it readily, although there are differences in pathogenicity of different races.

Helminthosporium Z resembles *H. carbonum* but differs in size of conidia and in pathogenicity on certain hosts, especially teosinte. It may be a new species of *Helminthosporium* or a variety or race of *H. carbonum*.

The host range differs for the several species of *Helminthosporium*. *H. sativum* has the widest host range. *Helminthosporium* Z infected Sudan grass in addition to corn, whereas it did not attack teosinte readily. *H. carbonum* infected teosinte severely; and *H. turcicum* infected sweet corn, teosinte, and Sudan grass. Varieties of corn and teosinte react differently to species and races of *Helminthosporium*.

Helminthosporium carbonum, *H. turcicum*, and *H. sativum* each comprise two or more parasitic races.

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FURTHER STUDIES OF THE BACTERIAL NECROSIS
OF THE GIANT CACTUS[1]

ALICE M. BOYLE[2]

The first report on the bacterial necrosis of the giant cactus (*Carnegiea gigantea* Britt. and Rose (*Cereus giganteus* Engelm.)) was published in 1942 by Lightle, Standring, and Brown (10). Since that year the toll taken by the disease has been great in the Saguaro National Monument, the Tucson mountain area, the Catalina Foothills, and in plantings on estates in the region where the giant cactus grows. Scenes such as shown in figure 1, A have become increasingly common. Thus, because of its economic as well as its botanical importance, studies of the disease have been continued.

REVIEW OF PREVIOUS WORK

The first symptom is a small, circular, light-colored spot, usually with a water-soaked margin, anywhere on the surface of the integument of trunk and branches (10). Parenchymatous tissues under the integument first appear water-soaked, but soon turn brown to almost black. As the infection progresses, the spots on the surface enlarge and become purplish. In some cases the integument may break and a brown liquid may exude. Slower decay of the tissues may go on without "bleeding," but the rotted tissues dry, break up into a granular, lumpy mass, and fall to the ground leaving the woody stelar strands bare.

Histologic and cytologic changes due to the bacterial invasion were discussed (10). Drying, cracking, and discoloration of the cuticle-covered epidermis and the hypoderma take place. The chlorenchyma loses its chlorophyll, becomes water-soaked, and later is discolored brown to black; all other parenchymatous tissues also appear water-soaked and later are discolored. Finally the soft tissues break down into a dark-colored mass. Cytologic changes leading to the breakdown of parenchyma tissues affect the entire cell. The wall loses its middle lamella by dissolution and eventually becomes entirely soft and irregular in outline. Early in the progress of the

infection, the nuclear membrane thickens; the nucleoplasm loses its reticular aspect and becomes more or less homogeneous and retentive to stains; increase in bulk of the nucleus may be followed by shrinking, its outline becomes irregular and wrinkled, and disintegration follows.

Inoculation experiments were reported (10), showing a bacterium to be the cause of the giant cactus necrosis. Characteristics of the bacterium were given and the name *Erwinia carnegieana* Standring was proposed.

The disease is distributed over an area coinciding with the habitat of the giant cactus in the United States—an area 200 miles broad, north and south, by 250 miles long, east and west. The disease has been particularly destructive near Tucson, in Picacho Park, and near Maricopa, Arizona.

SPREAD OF THE DISEASE

Rapid spread of the bacterial necrosis of the giant cactus may be accounted for in part by infection of a healthy plant from a diseased one by way of closely associated root systems. The plants often grow very close together in groups. If one plant in such a group becomes diseased, almost invariably others of the group also succumb, lesions very often developing near the base. Over a period of years, one plant after another in the group is infected, so that eventually as many as ten or twelve giants in a cluster have been killed.

Erwinia carnegieana was isolated in 1944 from the exposed roots of a plant weakened by extensive basal lesions and blown over in a windstorm. Since that time several infected root systems have been observed and the roots cultured. This condition has been induced by inoculation (Fig 5, C and D).

A "bleeding" cactus depositing exudate on the ground may be a source of infection to healthy cacti nearby. The exudate may consist of several gallons of almost black liquid which contains the bacteria that cause the disease. The exudate is often of sufficient quantity to soak the soil to a depth of 3 or 4 in. in a wide area about the diseased plant. Since many roots of the giant cactus are very close to the surface of the ground or partly exposed and have cortex broken by action of weather, rodents, or insects, infection of the roots of plants growing near an infected one is possible.

The bacterium *Erwinia carnegieana* is capable of living in the soil and there remaining viable for considerable time. Soil which was soaked with the exudate from a "bleeding" cactus was collected during the summer of 1945 and brought into the laboratory for study. Isolations were made and cultures of *E. carnegieana* obtained. The soil was then divided into two parts. One part was allowed to dry, and to the other was added enough water from time to time to keep it in about the same condition of moisture as when collected. At frequent intervals, varying from 5 days to a week, soil samples were taken from the two lots and isolations were made by the usual dilution method. After 46 days of exposure to Tucson's summer temperature and to drying in one case, *E. carnegieana* was isolated from both

lots of soil.   The added moisture was found not to affect the length of time
the bacterium remained alive in the soil, so it is not probable that summer
rains would extend the life of *E. carnegieana* in the soil. Figure 1, C shows
a cactus inoculated with the bacterium isolated from the soil 46 days after
collection of the soil sample.
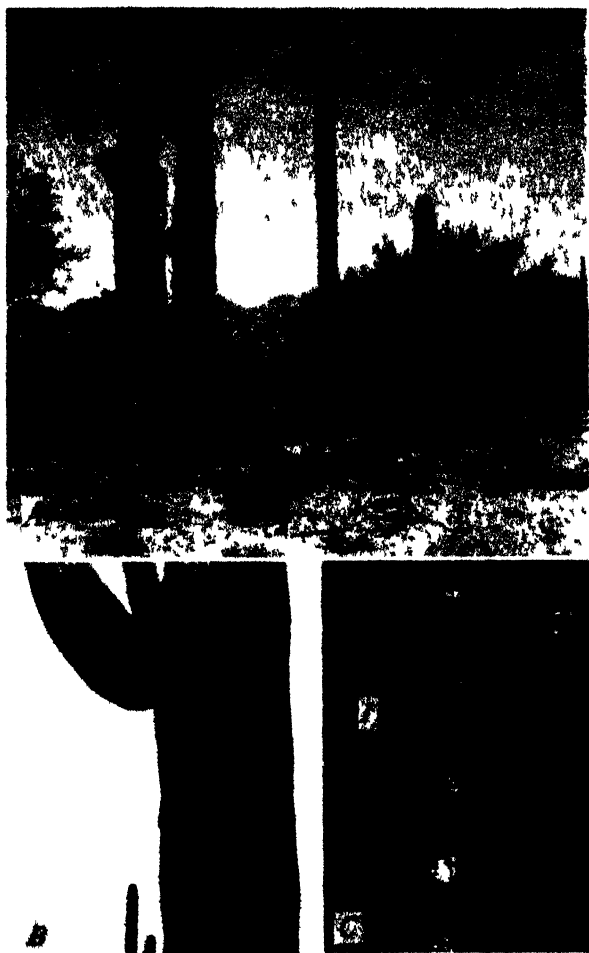
FIG 1. A. Destruction of *Carnegiea gigantea* caused by *Erwinia carnegieana*, a
frequently occurring sight in many giant cactus areas   B. Necrotic branch of giant
cactus which caused infection of trunk at point of contact   C Plant inoculated with
E. carnegieana isolated from soil 46 days after collection   Inoculation point at *ι.*

The two means of infection discussed may account to a great extent for
the basal lesions that are so often fatal.   Rather extensive lesions at the
top of a giant cactus or on branches may not necessarily prove fatal to the
plant.   When a giant cactus is girdled at the base, however, it is so weak-
ened that it easily breaks off in a windstorm.   Such broken giants (Fig. 1.
A) are a common sight in forests of giant cactus.

A plant or plant part may be infected directly in cases where a diseased branch comes in contact with a healthy trunk or branch. A diseased branch of giant cactus may become weakened and fall against a healthy part of the trunk of the same individual (Fig. 1, B) or even another growing near by. The movement of the diseased branch by the wind may injure the healthy tissues so that the parasite is able to enter the latter. In the case of the saguaro photographed, a lesion about 6 in. in diameter has developed on the trunk during the 8 months the branch has been dangling and striking against it in the wind.

By far the most important factor in the spread of the cactus necrosis, however, is the insect vector.

THE INSECT VECTOR

The opinion that an insect vector was chiefly responsible for the spread of the bacteria from diseased cacti to healthy ones was held by Lightle and co-workers (10). Tunnels, apparently made by insects, had been found associated with necrotic lesions. A study of the insects frequenting the giant cactus was undertaken by the present author. Because such a wealth of insect life is found in and on the plant (8, 9), many insects were collected, cultured, and reared, and their life histories studied in relation to the giant cactus before the vector was determined.

Prior to 1942, the giant cactus necrosis had been controlled (6) by removal and burial of badly diseased plants.

In the spring of 1942, the control work on the disease, which was being continued in the Saguaro National Monument by Dr. James Mielke, in the absence of Dr. Gill, afforded an opportunity for observation and study of the insects found in giant cactus. There was available a wealth of infected material with tunnels such as had been occasionally observed in the soft tissues of plants. · The tunnels varied in direction and size. Careful dissection of tunneled plants showed "rot pockets" or lesions developing at random along the length of the tunnels, both internal and external to the stele of the plant (Fig. 2, A and D). *Erwinia carnegieana* was isolated repeatedly from tissue surrounding the tunnels.

In June 1942 the first larvae responsible for the tunneling were found in giant cactus (Fig. 2, C). When placed in a jar with fresh cactus tissue, larvae tunneled readily and rapidly directly through the cutinized epidermis and hypoderm as well as through the softer tissues (Fig. 2, B). *Erwinia carnegieana* was isolated from the surface and from the intestinal tracts of the larvae. In the latter case, specimens were surface-sterilized in 1 : 1000 mercuric chloride and crushed on agar plates.

Some of the larvae were successfully reared in wire cages in the laboratory, and the bacterium responsible for the necrosis of the giant cactus was isolated from the adult and from the surface of the eggs. In April, 1944, after inoculation experiments had shown the insect capable of transmitting the disease, adult specimens as well as larvae and pupae were sent to the

U. S. Bureau of Entomology where the insect was identified by Mr. Carl Heinrich as *Cactobrosis fernaldialis* (Hulst) (8).

Observation in the field and laboratory from 1942 to the present time have led to the conclusion that *Cactobrosis fernaldialis* has but one genera-

FIG. 2.   A. Longitudinal section of branch of giant cactus showing tunnels made by larvae of *Cactobrosis fernaldialis*.   B. Larvae of *C. fernaldialis* at *l* and tunnels made by them at *t*.   C. Close-up view of *C. fernaldialis* (*l*) in a tunnel made by it in giant-cactus tissue.   D. Cross section of branch showing tunnels made by larvae.

tion a year.   The larvae have been found tunneling in giant cactus from November 2 until August 20, so it is apparent that the longest period of the insect's life span is its larval stage when it feeds in the giant cactus.   The larvae are never found in tissues that are in a late stage of disintegration caused by *Erwinia carnegieana*, but rather constantly move away from such

FIG. 3.   A. Surface view of giant cactus showing emergence hole through which larva has left plant to pupate (*t*).   B. *Cactobrosis fernaldialis* adult and pupa case containing pupa.   C. Pupa of *C. fernaldialis* near emergence hole on surface of a giant cactus.   Emergence hole lies in the shadow of the pupa.   D. Eggs of *C. fernaldialis* on wire mesh of breeding cage.

areas as the disease advances into healthy tissue.   From May through August, when larvae leave the plants to pupate, fresh, uncorked emergence holes are frequently seen (Fig. 3, A).   Although the insects occasionally pupate on the plant near the emergence hole (Fig. 3, C), it is characteristic

of them to seek protection under fallen pieces of cactus, stones, or sticks. When the moths were reared in captivity the larvae never pupated in the open but always on the underside of available debris such as pieces of wood, cactus, or other objects.

The pupal stage is from 28 to 33 days' duration. The adult nocturnal moth, light tan with dark brown markings (Fig. 3, B), is short-lived, laying its eggs and dying within three days. This observation agrees with the report of Dr. Carl Heinrich on the life history of the insect (8). The eggs have been found in nature only twice, borne singly on a cactus spine near the apex of a low-hanging branch. In breeding cages they are seldom deposited on cactus plants placed in the cages but most often are deposited singly and in great numbers on the wire mesh of the cage (Fig. 3, D). During the 30-day period before hatching, the tiny eggs change color from their original cream through deepening shades of pink to a rather brilliant red. During the first few hours after the very small larvae emerge from the eggs, their mortality rate is high. They move around apparently aimlessly, but roughly about 10 per cent of the larvae reach a cactus plant, entering either directly through the unbroken cuticle and epidermis or, more often, by way of the softer tissues at the growing point.

Inoculations.—Since it was obviously impractical to build cages large enough to accommodate mature giant cacti, the plants used in these experiments were either very young ones grown from seed in the greenhouse and kept in cages 12 × 12 × 18 in., or were plants 1 to 2 ft. high transplanted from the cactus forests to pots and placed outdoors in cages 4 × 4 × 5 ft. The small, greenhouse-reared plants are not heavily cutinized, hence the ¼-in. larvae were capable of entering at any point. The larger cacti used were never entered except near the apex, and it is believed that in nature the larvae enter the plant through the soft tissues at the growing point. In dissected plants, both the larger ones mentioned above and those examined in the field, narrow tunnels near the trunk and branch tips appear to have been made by small larvae. As the distance from the plant apices increases, the tunnels widen, appearing to have been made by the larvae after they have fed and grown within the plant. The larger and therefore older steel-gray larvae may eat out a tunnel ¼ in. in diameter and many yards in length.

The period during which larvae are entering the cactus plants coincides with the 150-day period of growth at the trunk and branch apices reported by Macdougal (12). Within 10 days after larvae have entered plants, in a high percentage of cases, typical symptoms of the necrosis are apparent (Fig. 4, A and B).

Plants too large to place in the insect breeding cages also were inoculated with larvae. On January 27, 1944, eight larvae were placed in a cavity made by cutting out a plug about 3 in. in diameter at the epidermal end and extending, wedge-shaped, 5 in. into the plant. An inch was cut off the small end of the wedge, the larvae were inserted into the hole in the plant, the wedge was replaced and the cut sealed firmly with beeswax. Figure 5,

A shows the resulting lesion on February 24, 1944. "Bleeding" had started
3 days earlier, indicating that extensive breakdown of tissues had taken
place, undoubtedly because of bacterial invasion since symptoms were iden-
tical with known symptoms of the disease in nature. By April 26, 1946,
progress of the parasite had been arrested by the formation of layers of
cork separating the lesion from healthy tissues (Fig. 5, B). A large cavity
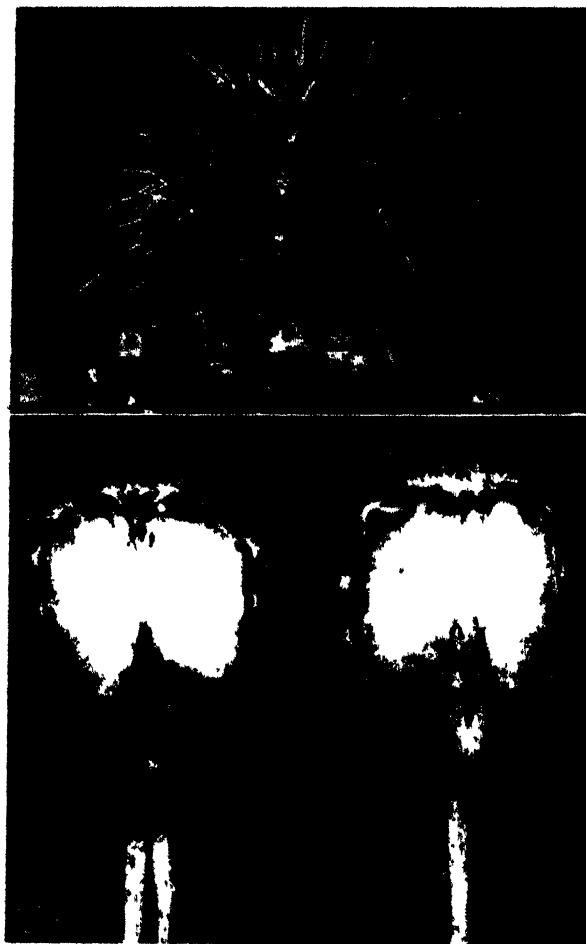
FIG. 4. A. Giant cactus 2 in. high showing necrosis at n as a result of invasion by
larvae. B. The same plant sectioned to show tissue discolored due to necrosis.

extended almost to the woody cylinder. Whether the advance of the dis-
ease has been checked permanently is not yet certain. The plant is still
under observation.

Another giant cactus was inoculated at the same time and in the same
way, but at a point just above the ground line. The only noticeable symp-
tom was a gradual loss of the normal dark green color of the entire plant,
until the plant became pale yellowish-green. This condition has often been

observed in nature before any lesions, "bleeding," or other local symptoms
appear.   Such plants, after a long time, give evidence of the extensive necro-

FIG. 5.   A. "Bleeding" of lesion resulting from inoculation by larvae of *Cacto-
brosis fernaldialis.*   B. Giant cactus shown in A, 2 years after inoculation   this plant
received over twice the normal amount of water.   C. Natural appearance of giant cactus
killed by inoculation with larvae of *C. fernaldialis.*   Base of plant with rotted root sys-
tem and extensive basal lesion.   D. Longitudinal section of giant cactus shown in C.
Larvae of *C. fernaldialis* were inserted in healthy plant approximately at *i;* *w* shows
infected, water-soaked tissue; *d,* decomposed tissue.

sis which has been progressing within them. And so it was with the inoculated plant under discussion. The plant was photographed March 12, 1948, a few days after it had blown over in the wind (Fig. 5, C). The root sys-
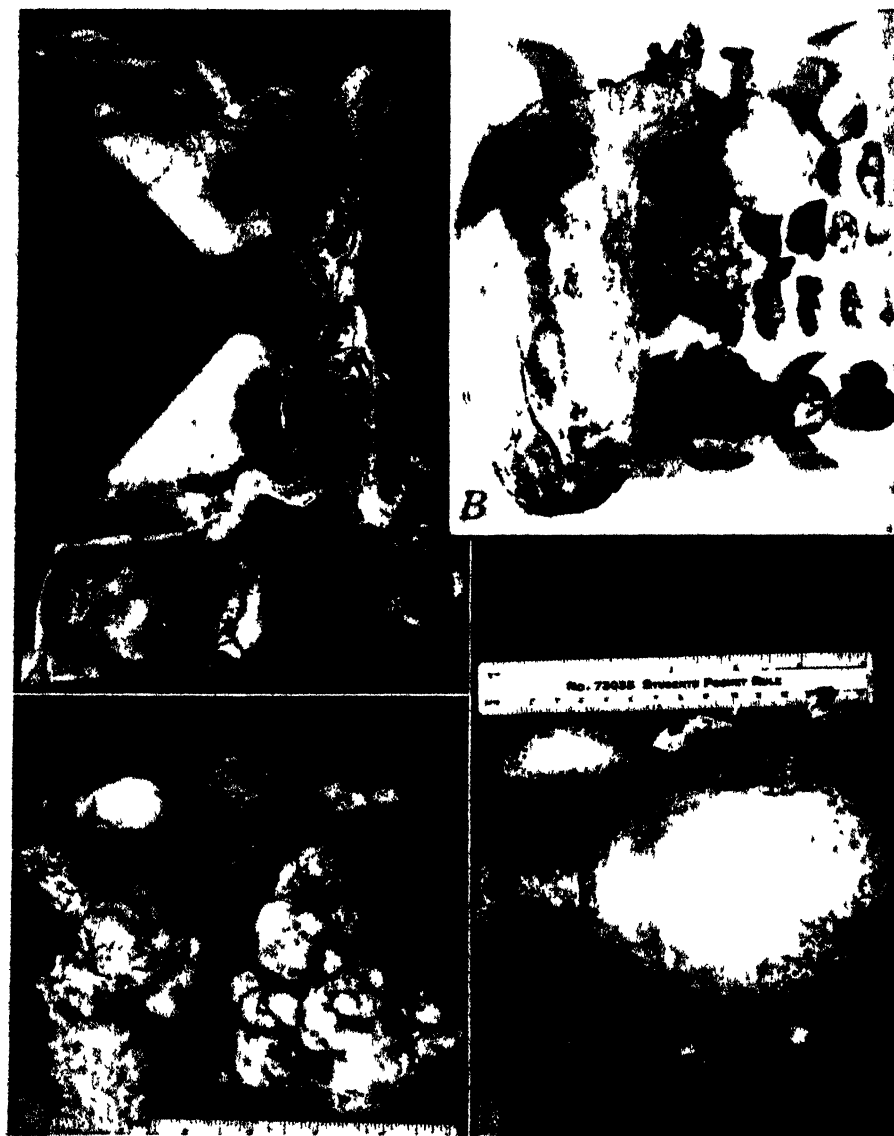
FIG. 6.   A. Cone-shaped, suberized structure, split through the center to show layers of corky cells with strands of parenchyma cells between. B, C, and D. Cork-structures which developed around necrotic lesions in soft tissues of giant cactus.

tem was almost entirely rotted away, and the plant was girdled by a lesion at the base. In longitudinal section (Fig. 5, D) the entire base of the plant has the typical discoloration and softening.

Corking-off.—The "corking-off" process, which is one means of defense against invasion of the parasite, is common in nature as well as in experimentally inoculated plants. Formation of cork is stimulated as readily by any injury as by the invasion of the bacterium responsible for necrosis. Figure 6, A shows a cone-shaped, suberized structure enclosing an infected area, split open through the center to show layers of corky cells with strands of parenchyma cells. The white dots are crystals. The dark areas enclosed by the corky layers still contained living bacteria capable of progressing into the healthy tissues should breaks occur in the enclosing shell.

In many cases of natural infections and also those produced by inoculations, the corking-off does not keep pace with the rapid progress of the pathogen, or breaks through the cork layers occur and, finally, total destruction of the plant results. Peculiarly shaped cork structures are often found on the ground where a giant cactus has fallen and disintegrated, indicating that the cactus had repeatedly resisted the attack of the parasite by cutting off its progress temporarily by means of layers of suberized cells, and the parasite has just as often broken through these layers. Figures 6, B, C, and D. show that these cork structures may assume practically any size or shape, depending upon that of the lesion outlined. Tubular corked structures, the result of the corking-off of tunnels, are sometimes so extensive in length and branching and so thin-walled that they cannot be preserved sufficiently intact to be photographed.

Other Insects

Some of the insects other than *C. fernaldialis* found associated with necrotic lesions may play a part in the progress of the disease. Larvae of *Volucella* species are found often by the thousands lining a lesion on the surface between healthy and infected tissues. A flat, black beetle identified as *Hololepta yucateca* (Marseul), predacious on saprophagous insects, is usually found. A small black beetle and its larva are numerous, as is also a large larva of an unidentified moth. These larvae and adult insects have been found only in the disintegrated tissues of the plant, not progressing into normal tissues as does *Cactobrosis fernaldialis*. *Erwinia carnegieana* has been isolated from body surfaces but never from the intestinal tracts of any insect except *C. fernaldialis*. It is probable that some spread of the disease is accomplished by them, and their constant activity and presence in such large numbers may help to prevent the normal corking-off of the plant about a lesion, or may help cause breaks in the cork tissue facilitating the progress of bacterial invasion.

AGE OF THE GIANT CACTUS AND SUSCEPTIBILITY
TO BACTERIAL NECROSIS

In 1942, when work was first started by the author, the number of old plants killed by the disease was noticeably so great that a relationship between age of plants and susceptibility to necrosis was considered a possibility. Since that time, so many naturally infected young plants have been found (Fig. 7) and inoculations have produced the disease in young plants

in such a high percentage of cases (Fig. 4, A and B), that this supposition has now been abandoned. The higher percentage of deaths of older plants can be adequately explained by the fact that they outnumber young ones. In some areas, notably the Saguaro National Monument, the percentage of

FIG. 7.   A giant cactus 18 in. high showing a lesion resulting from natural infection with *Erwinia carnegieana*.

young plants is very small. It is difficult to find plants less than 10 ft. in height in this area. In the Tucson mountain area and in the Catalina Foothills, plants of all sizes are more plentiful but young ones are still very much in the minority.

The lack of young plants may be due to several factors. Flowers become infected with the rot bacterium and fail to produce seeds. *Erwinia carnegieana* has been isolated from partly rotted flowers and immature fruits collected from the ground where they had fallen. A similar condition has been produced experimentally by spraying blossoms with a bacterial suspension of *E. carnegieana*, and this bacterium has been reisolated from the rotted flowers. According to Benson and Thornber (2), and the writer's record of the flowering period of the giant cactus, this period coincides with that during which the young larvae of *Cactobrosis fernaldialis* are entering the plants. Although larvae have never been found in the partly rotted flowers and fruits collected, tiny holes thought to be made by the larvae have been observed.

This, then, accounts for a reduction in seed production. Nevertheless, a high percentage of flowers do produce mature fruits. As a result of overgrazing, ground cover in many of the cactus areas has been so greatly reduced that no protection is offered the fruits when they drop to the ground and many are consumed by rodents or birds. Some escape this fate to germinate and produce small plants which, unprotected, are also eaten by rodents. The result is a very few young plants which happen to start life in a clump of mesquite or other desert shrub.

H-ION CONCENTRATION OF CELL SAP OF GIANT CACTUS
AND ITS RELATION TO BACTERIAL NECROSIS

Changes in H-ion concentration of the sap of plant tissues as a result of disease are expected. Fischer and Gaümann (4, pp. 368–371) give an excellent review of the literature on this subject. Examples are given to show changes in pH toward the acid side with infection by one parasite, either bacterial or fungus, in a certain host, and a change toward the alkaline side with a second parasite in the same host. Also the same parasite may cause changes in tissue liquids of two different hosts, in one case toward the acid side and in the other toward the alkaline. The conclusion, then, would be that such changes in pH of plant tissues due to invasion by a parasite are the sum of the reaction of the host to the parasite and of substances given off into the plant by the parasite.

Gaümann (5, pp. 322–323) discusses the fact that pH is often a limiting factor in invasion of plant tissues by a parasite.

Using a Beckman pH meter in the field, pH readings were taken on cactus tissues at different seasons of the year. Pieces of plant tissues to be tested were removed from the plant, the sap pressed out with a small hand press, and the test made immediately. The following results were obtained: apparently uninfected plants, pH 5 to 5.5; healthy-appearing tissue of infected plants, 7 to 7.4; discolored tissue not yet broken down, 8.7 to 9; soft, broken-down tissues, 3 to 3.5.

The readings for healthy tissues agree with those of Macdougal (12). The difference between the pH of uninfected plants and healthy-appearing

infected plants was first thought to be due to a genetic difference in the plants, making the latter more susceptible to the disease. *Erwinia carnegie-ana* was tested in broth cultures adjusted to pH from 3.0 to 8.0. A pH of 7 to 7.2 is optimum for its growth, and very little growth takes place at pH 5 to 5.2 and none below pH 5. However, pH readings taken on noninfected 5- and 6-year-old plants grown in the greenhouse from seed were in exactly the same range as that of older plants. Dr. Macdougal's work (12) has shown no differences in pH of giant cactus tissues due to age. Since the plants tested were grown from seed of infected as well as healthy plants, it is probable that a difference in pH would have been apparent had any genetic difference been present. No attempt has been made to explain changes in pH of the infected tissues since chemical analyses have not been made.

RAINFALL AND BACTERIAL NECROSIS OF THE GIANT CACTUS

In an effort to determine whether amount of rainfall influences the progress of giant cactus necrosis, eleven healthy giant cacti were transplanted in April 1944 to the University of Arizona campus. These were used for inoculation work and experiments concerning water relations.

TABLE 1.—*Monthly additions of water to five of eleven* giant cactus plants studied at Tucson, Arizona, in 1945

Month	Average rainfall[b] 1875–1944 (inches)	Rainfall 1945 (inches)	To attain average		Excesses		
			Plant No.		Plant No.		
			5	9	3	7	11
January	0.42	0.65	0.00	0.00	0.42	1.40	0.42
February	0.94	0.41	0.53	0.53	1.47	2.00	1.47
March	0.77	0.62	0.15	0.15	0.92	2.01	0.92
April	0.35	0.08	0.27	0.27	0.97	1.00	0.97
May	0.19	0.00	0.19	0.19	1.90	2.90	1.90
June	0.27	0.00	0.27	0.27	1.35	2.40	1.35
July	2.16	1.22	1.14	1.14	2.16	2.50	2.16
August	2.29	2.95	0.00	0.00	2.29	2.52	2.29
September	1.19	0.12	1.05	1.05	1.26	1.50	1.26
October	0.26	1.42	0.14	0.14	1.42	1.50	1.42
November	0.80	0.00	0.80	0.80	1.60	2.00	1.60
December	1.07	0.16	0.91	0.91	1.98	2.00	1.98
Total	11.41	7.63	4.73	4.73	17.74	23.73	17.74

a No additions to plants Nos. 1, 2, 4, 6, 8, and 10.
b Rainfall data for comparison, obtained from weather station located approximately 200 ft. from row of giant cacti studied.

As will be seen by referring to table 1, the average rainfall for the years 1875 to 1944 was taken for a basis. Plants 2, 4, 6, 8, and 10 were given no additional water although the total rainfall for the year 1945, during which the tests were run, was below the average. Plants 3 and 11 were given 17.74 in. water additional to the rainfall for the year, or a total of 25.37 in., more

than twice the average rainfall.   Plant 7 was given 23.74 in. water in addition to the rainfall, or a total of 31.37 in., nearly three times the average. Plants 5 and 9 were given just enough additional water to bring the total amount up to the average each month. Actually the total amount of water received by these giants was slightly in excess of the average because, although the total rainfall for 1945 was below average, that for the months of January, August, and October was slightly above.

After determining the amount of water in cubic feet to be added to the 6 × 6-ft. depressed areas around the giant cactus, a water meter was used to measure the additional water applied. At the end of each month when the figures on total rainfall for the month became available, the treatment was given.

The giant cacti were inoculated by different means—by the stab method, by larvae, and by spraying the flowers with a suspension of *Erwinia carnegieana*. No difference in speed of infection or progress of the disease was noted.

Plant 11 (Fig. 5, A and B) received more than twice the average normal amount of water; plant 1 (Fig. 5, C and D) received no extra water. Floral infection in plants 4 and 7 took place in about the same percentage of cases as did infection by the stab method in plants 3 and 9. Conclusions based upon results with so few plants cannot be final, but results are supported by later observations made in the field. The total rainfall for 1944 was 13.32 in., and for 1946, 13.68 in.; both above the long-time average. The year 1947 totaled only 5.72 in. There has been no substantial difference in the progress of the disease during these years, which seems to substantiate the conclusion, obtained experimentally, that amount of rainfall is not a contributing factor in the progress of the disease.

STUDIES WITH THE PATHOGEN

Cultural Studies

The bacterial cultures used in these comparative studies originated from fresh isolates that had been tested for pathogenicity. The tests were run at 30-day intervals over a 15-month period. Stock cultures were kept in the refrigerator under sterile mineral oil. Each month transfers were made to nutrient broth, and after 24 hr. were utilized as inoculum for all the media employed in the tests. Transfers were also made from the stock cultures to potato-dextrose-agar slants, incubated until good growth was evident and placed under sterile mineral oil in the refrigerator until the next series of tests was run about a month later. The Manual of the Society of American Bacteriologists (13) was followed in these tests.

Durham fermentation tubes were used for all fermentation studies. Bacto-nutrient broth with 1 per cent carbohydrate added and brom cresol purple as an indicator were used throughout.

All plant-tissue agars were made by using 15 gm. agar, 20 gm. dextrose,

and 200 gm. plant material to the liter. The plant material was cooked in about 500 cc. of water until soft, the water drained off, and enough tap water added to bring the amount of liquid to 1 liter.

The steamed sweet potato, rice, and cornmeal were prepared by cooking the materials enough so that they could be smoothly spread in the bottom of a Petri dish to a depth of about $\frac{1}{4}$ in.; adding a small amount of water, and sterilizing in the autoclave for 20 min.

Other media were prepared according to the Manual of the Society of American Bacteriologists, except that spirit-blue agar which was used for tests of lipolytic activity was prepared according to Starr and Burkholder (17, 18).

Tests for indole and for nitrate reduction were carried out each time a series of cultures was run.

Gram stains and inoculations to determine possible changes in pathogenicity of the bacteria were also made. The necrosis bacterium was compared with four isolates of *Erwinia carotovora*: No. 1 from onion, No. 2 from garlic, No. 3 from spinach, and No. 4 from carrot.

Tables 2 and 3 show the results of the work at the beginning of the tests in May, 1943. At this time inoculations showed all the cultures to be strongly pathogenic, *Erwinia carnegieana* was Gram positive, and all the *E. carotovora* strains were Gram negative.

Tables 2 and 3 show the physiological and cultural changes resulting during the period from May to December, 1943, when transfer had been made and cultures run each month. By comparison it will be noted that *Erwinia carnegieana* and *E. carotovora* isolate No. 1 underwent comparatively few changes. Isolate No. 2, almost identical with *E. carnegieana* in May, still resembles it markedly. *E. carotovora* No. 4 changed very little during this period; the greatest number of changes occurred in isolate No. 3. No apparent decrease in pathogenicity of any of the bacteria could be detected up to this time.

Between December, 1943, and August, 1944 (Tables 2 and 3), a gradual decrease in vigor of growth in and on all media is noticeable. Most strains of *Erwinia carotovora*, but no known strains of *E. carnegieana*, lost the ability to utilize carbohydrates; exceptions were some of the di- and mono-saccharides. Pathogenicity was completely lost. *E. carnegieana* by this time retained gentian violet only slightly in Gram staining, so was more Gram negative than positive, while *E. carotovora* No. 2 became Gram positive.

Erwinia carotovora isolate No. 1 did not originally produce indole but acquired the ability by December, 1943. By the end of the testing period, indole was again not produced; isolate No. 2 lost its ability to produce indole and did not regain it. In this respect it differs from *E. carnegieana* which it closely resembled in May, 1947. *E. carnegieana* retained its indole-producing ability throughout the tests; isolate No. 4 lost and regained the

TABLE 2.—*Comparison of reactions of Erwinia carnegieana and E. carotovora in various carbon sources in May, 1943, December, 1943, and August, 1944*

Carbon source	E. carnegieana			Isolates of E. carotovora											
	May	Dec.	Aug.	1			2			3			4		
				May	Dec.	Aug.	May	Dec.	Aug.	May	Dec.	Aug.	May	Dec.	Aug.
Monosaccharides															
Arabinose	A+G	A+G	Alk	A+G	A+G	Alk	A+G	A+G	Alk	A+G	A+G	A+G	A+G	A+G	A+G
Dextrose	A+G	A+G	A+G	A+G	A+G	A+G	A+G	A+G	A+G	A+G	A+G	A+G	A+G	A+G	A+G
Fructose	A+G	A+G	A+G	A+G	A+G	Alk	A+G	A+G	Alk	A+G	A+G	A+G	A+G	A+G	Alk
Galactose	A+G	A+G	Alk	A+G	A+G	Alk	A+G	A+G	Alk	A+G	Alk	Alk	A+G	A+G	Alk
Levulose	A+G	A+G	Alk	A+G	A+G	Alk	A+G	A+G	Alk	A+G	Alk	Alk	A+G	A+G	Alk
L. xylose	A	A	Alk	Alk	Alk	Alk	A+G	A	Alk	Alk	Alk	Alk	A+G	Alk	Alk
Rhamnose	A+G	A+G	Alk	A+G	A+G	Alk	A+G	A+G	Alk	A+G	A-G	A+G	A+G	A	Alk
Xylose	A	A	Alk	A+G	A+G	Alk	A+G	A	Alk	A+G	Alk	A+G	A+G	A	Alk
Disaccharides															
Lactose	Alk	Alk	Alk	A+G	A+G	Alk	Alk	Alk	Alk	A+G	A+G	A+G	Alk	Alk	Alk
Maltose	A+G	A+G	A+G	A+G	A+G	Alk	Alk	Alk	A+G	A+G	A+G	A+G	Alk	Alk	Alk
Melibiose	A+G	Alk	Alk	A+G	A+G	Alk	A+G	Alk	Alk	A+G	Alk	Alk	A+G	A+G	Alk
Sucrose	A+G	A+G	A+G	A+G	A+G	A+G	A+G	A+G	A+G	A+G	A-G	A+G	A+G	A+G	A+G
Trisaccharides															
Melezitose	Alk	Alk	Alk	A	A	Alk	A	Alk	Alk	A	Alk	Alk	A	A	Alk
Raffinose	A+G	A+G	Alk	A+G	A+G	Alk	A+G	A+G	Alk	A+G	Alk	Alk	A+G	A+G	Alk
Polysaccharides															
Glycogen	A+G	A+G	Alk	A+G	A+G	Alk	A+G	A-G	A+G	A+G	A+G	A+G	A+G	A+G	Alk
Inulin	Alk	Alk	Alk	A	A	Alk	A+G	Alk	Alk	A	Alk	Alk	A+G	A+G	Alk
Alcohols															
d-Sorbitol	A+G	Alk	Alk	A+G	A+G	Alk	A+G	Alk	Alk	A+G	A	A	A+G	A+G	Alk
Dulcitol	Alk	Alk	Alk	A+G	A+G	Alk	Alk	Alk	Alk	A+G	A+G	Alk	Alk	Alk	Alk
Inositol	A+G	Alk	Alk	A+G	A+G	Alk	A+G	Alk	Alk	A+G	Alk	Alk	A+G	A+G	Alk
Mannitol	A+G	A+G	Alk	A+G	A+G	Alk	A+G	Alk	Alk	A+G	Alk	Alk	A+G	A+G	Alk
Glucosides															
Aesculin	Alk	Alk	Alk	Alk	Alk	Alk	Alk	Alk	Alk	Alk	Alk	Alk	Alk	Alk	Alk
Salicin	A+G	A+G	Alk	Alk	Alk	Alk	A+G	A+G	Alk	Alk	Alk	A	A+G	A+G	Alk

* Alk = alkaline; A = acid; G = gas.

TABLE 3.—Growth of Erwinia carnegieana and four isolates of E. carotovora on media May, 1943, December, 1943, and August, 1944

Medium	E. carnegieana			Isolates of E. carotovora												
				1			2			3			4		
	May	Dec.	Aug.	May	Dec.	Aug.	May	Dec.	Aug.	May	Dec.	Aug.	May	Dec.	Aug.
Fermi's solution	Sl	N	N	N	N	N	Sl	Sl	N	N	N	N	N	N	N
Cohn's solution	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Endo's agar	A, MS	A, MS	A, MS	A, MS	A, MS	F, MS	A, MS	A, MS	A, MS	A, MS	A, MS	A, MS	A, MS	A, MS	F, MS
Malt extract agar	A	A	F	F	F	N	A	F	F	A	F	F	A	A	F
Simmons' citrate agar	Sl	N	N	N	N	N	Sl	N	N	Sl	Sl	F	F	A	F
Giant cactus agar	A	A	F	Sl	Sl	F	Sl	Sl	Sl	Sl	F	Sl	F	F	Sl
Asparagus agar	A	A	F	A	F	F	F	A	F	F	A	F	A	A	F
Carrot agar	A	A	F	F	F	F	A	F	F	A	A	F	F	A	F
Spinach agar	F	F	F	F	F	Sl	F	F	F	A	A	F	A	A	F
String-bean agar	F	F	F	F	F	Sl	A	A	F	A	A	F	F	F	F
Sweet-potato agar	A	F	F	A	A	F	A	A	F	A	A	F	A	A	F
Steamed sweet potato	A	A	F	A	A	F	A	A	F	A	A	F	A	A	F
Steamed rice	A	A	F	A	A	F	A	A	F	A	A	F	F	F	F
Steamed cornmeal	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F

a Sl=slight; F=fair; A=abundant; N=none; MS=metallic sheen.

ability to produce indole and lost its nitrate-reducing ability entirely. This ability was retained by all other strains of *E. carotovora* and by *E. carnegie-ana*. No hemolytic action or lipolytic activity was shown by any of the organisms throughout the test.

The evidence presented as a result of these tests indicates that *Erwinia carnegieana* belongs to the soft-rot group of bacterial plant pathogens. The group is an extremely variable one, and consequently results of cultural and physiological tests depend upon age of the culture and, in the case of strains of *E. carotovora*, the host from which the bacterium was isolated.

Agglutination Tests

Immunologists in the field of animal pathology have used plant materials to study the characteristics of plant proteins as antigens (21). The first use of the technique in the botanical field was for the purpose of detecting adulteration in flours and meals and foreign seeds in seed stocks. Later these methods were applied to genetic studies in separating and identifying strains and varieties of agricultural plants (23). The technique was applied to such mycological problems as the differentiation of varieties of yeasts and determination of relationship of certain fungi.

The first extensive piece of work dealing with a phytopathological problem was done by a soil bacteriologist interested in the bacteria of the nodules of legumes (24). The serological characteristics of strains were found to be definite, and to correlate with cross-inoculation of host plants. Stevens (19) made a careful study of 55 strains of nodule bacteria representing seven groups of cultivated legumes. His results confirm the earlier work as to the serological properties of the bacteria. Wright (22) found that the nodule bacteria of the soybean are distinct serologically, that serological characters are constant, and that they are unchanged by continued artificial culture or by plant passage.

Nelson (14) applied the agglutination test to a phytopathological problem. He found that he was able, by agglutination tests, to distinguish between the proteins of a flax resistant to wilt caused by *Fusarium lini* (*F. orysporum* var. *lini*) and a variety which was susceptible. Paine and Lacey (15) applied the agglutination test to a study of *Bacillus lathyri*, *Bacterium phaseoli*, and *Aplanobacter michiganense*. Although the first two gave group agglutination, the workers concluded that they could be differentiated by this method. *Aplanobacter michiganense* failed to agglutinate with serum of either of the other two. Takimoto (20) reported that serological tests indicate that the bacteria isolated from soft-rot of celery, lettuce, and radish belong to the same strain, although the radish organism differs from the others in culture. Goldsworthy (7) reported the formation of specific agglutinins, with a titre of 1 : 10,000 in rabbits following intravenous injections with *Bacterium maculicolum*, a cauliflower pathogen. He used these agglutinins to determine the presence of the bacterium in soils. Link and

Sharp (11) found that *Bacterium*[s] *campestre*, the crucifer pathogen, although distinct, is closely related to *Bact. phaseoli* and *Bact. phaseoli sojense* and less closely to *Bact. flaccumfaciens*. Their findings also show that biological specificity shown in their host relations is correlated with serological specificity. A. R. Stanley (16) reported on a study by the use of agglutination tests, of 120 cultures of soft-rot and colon bacteria. He reported that, although individuals of this large group were physiologically identical, some showed only a moderate agglutination, others showed no agglutination at all, and still others were thought to be identical serologically. The author also concluded that the soft-rot bacteria are closely related serologically to the colon-typhoid-dysentery group of bacteria. An extensive bibliography is given dealing with the use of serological methods in phytopathological work.

The object in using agglutination tests in the work with *Erwinia carnegieana* was to try to determine relationships between *E. carnegieana* and the strains of *E. carotovora* used in the cultural work described above. All freshly isolated bacteria were used in cultures which were tested for pathogenicity. The procedure followed was that given in the Manual of the Society of American Bacteriologists (13). A 24-hr.-old culture of *E. carnegieana* was injected intravenously into a rabbit, 2.5 cc. twice a week for 6 weeks. The serum obtained from the rabbit was employed in agglutination tests that were set up with the homologous strain and the four *E. carotovora* strains as antigen. The serum agglutinated the homologous strain completely at 1:10,000 and markedly at 1:20,000. No agglutination took place with any of the four *E. carotovora* strains as antigen. The work indicates that there is no antigenic relationship between *E. carnegieana* and the *E. carotovora* strains used. The use of a large number of soft-rot strains comparable to the number reported by Stanley in the paper quoted above might have shown relationships of "H" or "O" antigens. Antigenic analysis such as has been carried out with *Salmonella* group of bacteria might be of value in working out relationships in the soft-rot group.

Thermal Death Point

One marked difference between *Erwinia carnegieana* and *E. carotovora* is their thermal death points, found by the writer to be 59° C. in the case of the former, or 7° higher than that reported for *E. carotovora* (3).

CONTROL

Before the insect vector and its life history were known, control of the disease was attempted simply by opening up and cleaning out lesions which were recognizable in the plant by purplish-black areas on the integument. Cork was then quickly formed about the cavity, and although an unsightly hole resulted, this simple method was effective unless the plants were so tun-

[s] The genus *Bacterium* is now changed to *Xanthomonas*.

neled by larvae that numerous new diseased spots appeared later. Badly diseased plants were cut down and destroyed in an effort to reduce the source of infection or at least the amount of inoculum (6).

As knowledge of *Castobrosis fernaldialis* and its habits has grown, control of the insect has come to be considered the most promising means of control of the disease. Two per cent D.D.T. is not effective against adults or larvae of the moth. Tests were made by spraying insects kept in breeding cages.

Erwinia carnegieana was tested by the plate-penicylinder method (1), using crude penicillin made in the laboratory of the Department of Plant Pathology. It was found that penicillin had an inhibitory effect on *E. carnegieana* (Fig. 8, A) but none on *E. carotovora*. When commercial penicillin became available, the tests were repeated with the same results.

Although the use of penicillin in control work is not practical in large-scale operations, its application has been found effective in cases in which only a few plants are involved. The treatment does result often in a cavity, but not so unsightly a one as that left by digging out the decayed tissue. The results so far with plants treated by injecting penicillin into a lesion with a hypodermic needle indicate that the penicillin is able to diffuse through the plant tissues for a considerable distance. The plant pictured in figure 8, B showed at the time of treatment an unruptured purplish-black area about 4 in. in diameter at *i*. Almost a quart of crude penicillin was put into the lesion by plunging the hypodermic needle through the middle of the necrotic spot. As is often the case, the involvement of plant tissues was more extensive than appeared on the surface. Ten days after treatment the lesion had opened and was "bleeding" freely. Two weeks later the photograph was taken. No noticeable change took place in the ensuing three months. It is the opinion of the author that the holes above the site of treatment mark the site of another lesion present at the time of treatment but not yet apparent, and that diffusing of penicillin through the plant was sufficient to kill the bacteria present and check the progress of the disease. In order to come to a conclusion it would be necessary, of course, to collect data on treatment of a greater number of plants.

When lesions are detected and treated while still small, results are as seen in figure 8, C. The cactus illustrated was treated and photographed at the same time as the one shown in figure 8, B and has remained unchanged in appearance for the last three months. Of the twenty plants treated with penicillin, all except one show results similar to those photographed, varying in appearance with the extent of the lesion treated. The one exception was a plant with a discolored spot only 2 in. in diameter on the cutinized epidermis. Two weeks after treatment there was very little left of the plant but the woody stelar strands. Evidently involvement of tissues was extensive before the treatment was given.

FIG. 8. A. Effect of penicillin on *Erwinia carnegieana*. Antibiotic has diffused outward from cylinder and killed bacteria in the surrounding circular area. B. Giant cactus 24 days after treatment with penicillin: point of inoculation at *t*. Diffusion of penicillin evidently stopped bacterial action in infected tissues above point of inoculation. C. Small necrotic lesion treated with penicillin at *t*. Photographed 24 days after treatment.

SUMMARY

The previous work on the bacterial necrosis of the giant cactus is reviewed, and the rapid progress of the disease and its importance are emphasized.

Three possible methods of spread of the disease are discussed: contact of the root system of one infected plant with the roots of other individuals

in a group; saturation of the ground around an infected plant, thus furnishing inoculum for the spread of the disease to other plants through wounds in the roots; and by the insect vector, *Cactobrosis fernaldialis*, the most important factor concerned with the rapid progress of bacterial necrosis in the giant cactus forests of Arizona.

Certain factors and their relation to susceptibility to the disease and its prevalence are discussed. The possibility that there is a correlation between age, or between amount of rainfall, and mortality of giant cactus plants is not accepted. The role of insects other than *Cactobrosis fernaldialis* found in necrotic lesions is considered.

Studies concerning *Erwinia carnegieana*, the bacterium which causes the necrosis of the giant cactus, are reported. The result of cultural studies shows that *E. carnegieana* belongs to the soft-rot group on the bases of physiological and cultural differences. Agglutination tests show no antigenic relationship, however, between *E. carnegieana* and the four isolates of *E. carotovora* used in the tests. In addition to this difference, *E. carnegieana* is inhibited by penicillin and has a thermal death point 7° above that reported for *E. carotovora*.

Reference is made to the preliminary measures for the control of necrosis. Determination of the vector of the causal parasite and its life history should afford a scientific basis for more effective measures.

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PHYTOPATHOLOGICAL NOTES

Corticium Crown Rot of Gazania.—Gazania rigens, a common ornamental in California, is usually grown to serve as ground cover or as a border plant. Under conditions of high temperature, entire plantings or portions of plantings are killed by crown rot.

In 1948 almost all of the plants which were growing in flats in a nursery in Stanislaus County were severely affected by crown rot. The original stock was obtained by the nursery from a propagator whose stock was also
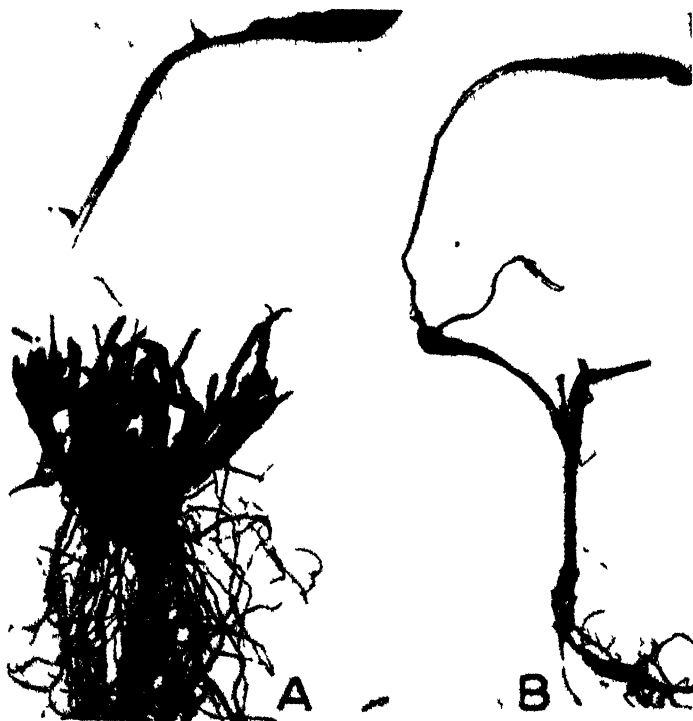
FIG. 1. Corticium crown rot of *Gazania.* A. A severely diseased *Gazania rigens* plant grown in the greenhouse. B. Cankers produced by *Corticium solani* on one of the shoots removed from the crown.

found to have the disease. The disease was also observed in three outdoor plantings in Alameda County.

The infection occurs at or below the ground line. The symptoms on the above-ground parts appear as wilting and withering of the leaves, beginning at the base and sometimes accompanied by twisting. The dead leaves and petioles become limp, fall to the ground, and easily become detached so that the diseased crown appears as a dark-brown to black mass of dead stems (Fig. 1, A and B).

Platings were made on acidulated potato-dextrose agar from diseased petioles and stems. After fungus mycelium could be seen under a binocular dissecting microscope, the tips of hyphae were transferred onto potato-dextrose slants and pure cultures were thus established. The fungus formed a sparse growth and produced sclerotia in culture in 2 to 3 weeks. Through the kindness of Dr. Byron Houston it was identified as *Corticium solani*. The optimum growth temperature for this strain was between 28° C. and 34° C. No growth was observed at 40° C.

For pathogenicity tests, the fungus was grown in Czapek's culture solution and only the mycelial mat was used in the tests. The mat was broken into small pieces in a Waring Blendor and poured over the surface of pasteurized soil (or mixed with it) in 6-in. pots in which healthy plants of *Gazania rigens* were growing. The plants inoculated with the fungus and kept outdoors during cool portions of the year showed no symptoms of the disease; but when these plants were transferred into the greenhouse at 28° C. and higher, wilting began after one week and the plants were completely destroyed in six weeks.

The relation of temperature to the fungus in culture closely paralleled that in soil either in the greenhouse or in the field. The disease has never been observed on plants grown under cool conditions, but is known to have been destructive in the warmer areas of the interior of the State.

Inoculations showed that *Gazania uniflora* and *G. splendens* are susceptible to the disease.—PETER A. ARK and ROBERT S. DICKEY, Division of Plant Pathology, University of California, Berkeley, California.

Botrytis Leaf and Blossom Blight of Saintpaulia.[1] The greenhouse culture of African violet (*Saintpaulia ionantha* Wendl.) has become an industry of considerable importance because of the demand for this flower as a houseplant. A leaf and blossom blight of African violet has occurred in the floricultural greenhouses at Michigan State College for some time. The symptoms, the causal organism, and suggestions for the control of this disease are discussed in this note.

Though no literature reference has been found of the occurrence of *Botrytis cinerea* Pers. on African violet, this fungus has been reported on such greenhouse-grown ornamentals as chrysanthemum and poinsettia,[2] geranium,[3] and cyclamen,[4] and on many ornamentals grown outdoors such

[1] Thanks are due to Evan Roberts, Supervisor of the Floriculture Greenhouses, for the Saintpaulia plants used in the course of these investigations and for supplying the initial infected Saintpaulia tissue, and to Clyde A. Bryant, Sr., Department of Horticulture, for aid in the original isolations and cross-inoculations.

[2] Spaulding, P. Botrytis as a parasite upon chrysanthemums and poinsettias. Mo. Bot. Gard. Ann. Rept., pp. 185–188. 1910.

[3] Melchers, L. E. Botrytis blossom blight and leaf spot of geranium and its relation to the gray mold of head lettuce. Jour. Agr. Res. [U.S.] 32:883–894. 1926.

[4] Hansen, H. N., and C. M. Tompkins. Cyclamen petal spot, caused by Botrytis cinerea, and its control. Phytopath. 38: 114–117. 1948.

as dahlia,[5] lupine,[6] rose,[7] and aster.[8] *Botrytis* has also been reported on a member of the Gesneriaceae by Baker,[9] who states that it causes a bud rot of *Sinningia*.

The disease usually first appears on *Saintpaulia* on the under side of the leaf petioles in the proximity of the pot rim. The first symptom is a small watersoaked lesion which may enlarge rapidly, circumscribing and extending lengthwise in the petiole, and on through the leaf blade. The necrotic area becomes brown to black with age.

The blossom-blight phase of this disease, which often originates in the throat of the corolla, produces premature fading and a watersoaked appearance of the corolla. If certain environmental conditions exist, *i.e.*, low light intensity, high humidity, and poor air circulation, the fungus may sporulate profusely on the infected tissue.

Infected corollas may drop on healthy leaves and thereby serve as a source of inoculum for the leaves. The disease has also been observed to spread from leaf to leaf where they are in direct contact with each other.

Isolations from diseased *Saintpaulia* tissue were made on potato-dextrose agar and have consistently yielded a fungus which has been identified as *Botrytis cinerea* Pers.[10]

The fungus produced conidiophores and conidia abundantly on oatmeal agar and potato-dextrose agar. Some conidia were produced on Czapek's medium, but very few on cornmeal agar.

Isolates of the fungus were pathogenic to *Saintpaulia ionantha* (Wendl.) and various named varieties such as "Blue Boy," "White Lady," "Blue Girl," "Plum," and "Pink Delight." The inoculum used was prepared by growing the fungus on potato-dextrose agar in Petri plates. After conidia were formed, sterile water was added and the spore suspension obtained was sprayed on the *Saintpaulia* plants. The plants were placed in chambers where the light intensity and relative humidity were optimum for growth of the plant. The blossom-blight phase of the disease became evident 4 days or more after inoculation, depending upon the environmental factors.

The fungus was readily reisolated from lesions that resulted from inoculations; it was grown in culture and when the spores were used to inoculate healthy plants it again caused typical expression of the disease.

Isolates of *Botrytis* of the *cinerea* type from peony, tulip, and geranium also caused characteristic symptoms of the disease when inoculated into

        [5] Cook, M. T., and C. A. Schwartz. A Botrytis disease of dahlia. Phytopath. 3: 171–174. 1913.
        [6] Weimer, J. L. Botrytis blight of lupines. Phytopath. 33: 319–323. 1943.
        [7] Smith, R. E. Botrytis and Sclerotinia: their relation to certain plant diseases and to each other. Bot. Gaz. 29: 369–407. 1900.
        [8] Gloyer, W. O. Fungous diseases of the China aster. (Abstr.) Phytopath. 14: 64. 1924.
        [9] Baker, K. F. Observations on some Botrytis diseases in California. U. S. Dept. Agr., Plant Disease Reptr. 30: 145–155. 1946.
        [10] The authors are indebted to Dr. C. J. Alexopoulos, Mycologist, Department of Botany and Plant Pathology, for the identification of the fungus.

Saintpaulia. Symptoms were indistinguishable from those caused by the isolate of *Botrytis cinerea* originally obtained from African violet tissue.

Undoubtedly one of the most efficient means of control for this disease is sanitation and adequate spacing of the *Saintpaulia* plants. Spacing allows ample air circulation and prevents the plants from touching each other. Good air circulation is very important, especially since authorities recommend low light intensity and a high relative humidity for the growth of the African violet. However, too high humidity and too low a light intensity, below approximately 450 foot-candles, should be avoided when practicable because these conditions favor the rapid spread of the fungus.

All diseased and dead tissue should be removed from the plants to prevent an accumulation of inoculum. The use of a sterile rooting medium for propagation and the use of "sterilized" potting soil is recommended to reduce the possibility of introducing inoculum from these sources. Care should be taken in handling the plants so as to avoid mechanical injury, thus limiting the number of avenues of entrance for the pathogen. The use of subirrigation might also aid in the control of the disease. Healthy, strongly growing plants are more resistant to *Botrytis* under many conditions than are weak, poorly growing plants.

Although no extensive search has been undertaken by the authors to ascertain the distribution of the disease, it can be assumed from conversation with scientists and growers that the disease may be widespread, and that it may appear wherever conditions are favorable. According to Dr. C. J. Alexopoulos, more than 20 years ago he noticed *Saintpaulia* plants in Illinois that had disease symptoms similar to those of plants which were inoculated with *Botrytis cinerea* by the authors. Professor R. A. Bowden, Department of Horticulture, stated that a disease of *Saintpaulia* not unlike the one described here is common in Georgia and that the use of glazed pots for growing the plants has reduced the occurrence of the disease there.

Because of the wide host range and prevalence of *Botrytis cinerea* it would be expected that the blossom and leaf blight of *Saintpaulia* may occur where conditions are favorable.—G. E. BECK, Department of Horticulture, and J. R. VAUGHN, Department of Botany and Plant Pathology, Michigan State College, East Lansing, Michigan.

Breaking of Cattleya Orchid Flowers by Orchid Mosaic Virus and its Transmission by Aphids.—A disease of Cattleya orchids which causes breaking in the color of the flowers (Fig. 1), distortion of the flowers, and mottling and malformation of the leaves has been noted by orchid growers in California for many years. Diseased plants typically become weak and stunted and produce progressively fewer flowers than normal.

The observations of some commercial growers in the San Francisco Bay region indicate that mosaic is present in some of the Cattleya plants imported from tropical America upon their arrival in California.

The writer has found no reference in the literature to a virus disease

of Cattleya orchids. There are, however, a few reports of diseases causing leaf mosaics in *Cymbidium*[1, 2, 3] and *Dendrobium*.[1, 4] Transmission has been reported only for Dendrobium mosaic and in this instance the disease was considered to be due to cucumber mosaic virus.[4] No statement has been published regarding the effects of mosaic on the flowers of *Cymbidium* and *Dendrobium*.

FIG. 1. *Cattleya mossiae* flower showing breaking in the color caused by orchid mosaic virus.

The present paper reports some of the results of experiments conducted during 1948 with a previously undescribed virus disease which causes a leaf mosaic and breaking in the flowers of *Cattleya* in California. The orchid plants used in these tests were *Cattleya mossiae* and *C. trianae* which are the species most commonly found to be diseased.[5]

Attempts to transmit the flower-breaking virus from orchid to orchid by

[1] Magee, C. J. Orchid mosaic. Australian Orchid Review 8: 51–52. 1943.
[2] Bissett, J. The black spot or mosaic in Cymbidiums. Australian Orchid Review 10: 48. 1945.
[3] Moore, W. C. Report on fungus, bacterial, and other diseases of crops in England and Wales for the years 1943–1946. Min. Agric. and Fish Bul. 139. 1947.
[4] Nobrega, N. R. Uma doenca de virus em orquidea. O Biologico 13: 62. 1947.
[5] The writer wishes to acknowledge his appreciation to N. W. Curson of Oakland, California, and to the E. W. McLellan Company of San Francisco, for donating the experimental orchid plants used in this study; and to Mr. Willis Sibray of the Alameda County Agricultural Commissioner's office, whose cooperation facilitated the investigation.

juice inoculation have been unsuccessful. However, the green-peach aphid, *Myzus persicae* (Sulzer), proved to be a vector of the disease. The aphids used in these tests were reared on sugar beets. They were first transferred to the unopened buds or open broken flowers of diseased orchids where they were allowed to feed for from 24 to 48 hr., and then transferred by brush to the test plants. In two cases the transfer was made by caging aphid-infested inoculum on the healthy test plants. The number of aphids per test varied from 100 to 300. Both winged and wingless forms were used.

In eight tests infective aphids were caged on healthy flower buds within a day after the buds emerged from the bud sheaths. Seven of these plants have developed mosaic symptoms. Five of the seven plants so infected developed some breaking in the flowers initially infected and also later developed leaf symptoms. In two cases the flowers fed upon developed no symptoms, but new leaves, produced on these plants since infection, have developed symptoms.

The time between infection of the buds and expression of breaking in the color of the flowers which developed from those buds was surprisingly short, ranging from 12 to 19 days. The amount of breaking was relatively small in most of these flowers, sometimes being confined to one or two sepals or petals. This would be expected because of the short time elapsing between infection and opening of the flowers.

The time required for leaf symptoms to appear in plants which had been infected through the flower buds varied from $2\frac{1}{2}$ to $4\frac{1}{2}$ months depending upon the growing condition of the plants. In each case the symptoms were delayed until a new shoot developed.

Infective aphids were caged on the young leaves of three orchid plants which carried no flower buds. Transmission was accomplished in two of these tests. The third plant has made little growth since inoculation and may yet develop symptoms when new growth is produced. The first plant infected through the leaf was *Cattleya trianae*. Symptoms first appeared on the inoculated leaf 32 days after infection. A new shoot arising from a different part of the plant began to show symptoms 40 days after the test was begun.

No transmission was obtained in three tests in which infective aphids were caged only on the flower bud sheath, nor in two tests in which the bud sheath and a maturing leaf were the only tissues available to the infective aphids.

The identity of the virus has not yet been established. However, the possibility of its being spotted wilt virus is precluded by the fact that it is transmitted by aphids and because mechanically inoculated juice from leaves and flowers of mosaic orchids failed to produce symptoms in garden nasturtium (*Tropaeolum majus*), which is highly susceptible to spotted wilt virus. Other host range experiments, including tests with plants known to be susceptible to cucumber mosaic viruses, have thus far failed to indicate virus hosts other than Cattleya.

Work is still in progress on the virus-vector relationships of the disease and on the search for additional hosts of the virus.—D. D. JENSEN, Division of Entomology and Parasitology, University of California, Berkeley, California.

A Qualitative Chemical Test for Some Stone Fruit Virus Diseases.— In the course of biochemical and physiological investigations of virus diseases, a chemical test was developed which has been helpful in the diagnosis and study of some of the stone fruit virus diseases.[1] During 1948 a simpler, qualitative test was devised which has proven to be a valuable diagnostic tool, both in the field and in the laboratory. Leaf tissue is generally used for testing, but when cherry fruit tissue is used, this test appears to be specific for the "little cherry" complex.

The chemical test appears to be a measure of the disease intensity; or, in other words, a measure of the intensity in reaction between a virus and the host. The chemical substance or substances that are responsible for the color reactions have not been identified but are presumed to be polyphenols.

A leaf sample, obtained with the use of a paper punch that cuts a disk of tissue $\frac{1}{4}$ in. in diameter, is taken from the middle portion of a leaf, either directly through the midrib or halfway between the midrib and the leaf margin. The midrib sample is the more sensitive. A single disk of leaf tissue is then placed in a small test tube with 1 ml. of 4 per cent sodium hydroxide. The tube is heated over a flame until the contents have boiled for about 15 seconds. The tube is allowed to cool for 30 sec., and is then shaken very vigorously in order to incorporate air with the solution. Oxygen from the air serves as an oxidizing agent to produce the colors. Pink or red indicates a positive test; green, a negative test.

With sweet cherries the milder virus diseases, such as the "ring-spot" or "latent" group, give varying shades of pink or light red. The stronger diseases, such as little cherry, rusty mottle, or twisted leaf, give a deep red color. With peach leaf tissue only the stronger diseases give a positive test. In Washington, Western X disease is the only peach virus disease that falls in this class.

Care should be taken in sampling to avoid leaves from branches that have been girdled. Reddened areas on peach leaves resulting from such disorders as nitrogen deficiency, arsenic injury, or Coryneum blight may also give misleading results. When the cherry leaf samples are taken, only spur leaves should be used.

Cherry fruit tissue may also be tested. The only cherry virus disease that has been found to give a positive fruit test is "little cherry"; thus it seems the procedure is specific for this disease. A cube of tissue about 4 mm. to a side is cut from the cheek of the fruit and treated like the leaf

[1] Lindner, R. C. A rapid chemical test for some plant virus diseases. Science 107: 17–19. 1948.

tissue. Any shade of pink or red is indicative of a positive test for "little cherry." Yellow or brown indicátes a negative test. The test works well on the fruit of the major commercially grown varieties of sweet cherries in Washington. The fruit, however, should be matured to the point that it is beginning to color or soften. Hard green fruit may give a false test. Fruit heavily infested with aphis, as well as fruit on a branch or spur that is girdled or infested with scale, should be avoided for samples. Certain Mazzard seedlings that produce small, bitter fruit also give a false test.

Samples of sour cherry fruit with typical "little cherry" symptoms give a distinctive red color when tested by this procedure. Occasionally a light pink is obtained with fruit that does not seem to have the "little cherry" disease. Since the "little cherry" virus is usually not uniformly systemic, comparative tests of healthy and diseased fruit from the same sour cherry tree should be made. A distinctive color difference between the healthy and diseased fruit samples indicates "little cherry."—R. C. LINDNER, Division of Plant Pathology, The State College of Washington, Tree Fruit Experiment Station, Wenatchee, Wash.; T. E. WEEKS, Washington State Department of Agriculture; and II. C. KIRKPATRICK, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

Pythium Black Rot of Cattleya.[1]—Black rot of orchids has been described recently by Rosetti[2] in Brazil where it damages species of *Laelia*. Inoculations of an unidentified phycomycetous fungus isolated from diseased specimens into wounded pseudobulbs produced the disease in *L. purpurata* Lindl. and Paxt. and *L. crispa* Reichb. f. Limber,[3] in 1946, in his report on the distribution of black rot of orchids, stated that what appeared to be black rot was found on Cattleyas from England, Colombia, and Venezuela.

The disease was found on seedlings as well as on mature, flower-bearing plants of *Cattleya* sp. in greenhouses in California. A survey showed the disease to be widely distributed and, in some instances, taking a rather heavy toll of *Cattleya Fabia* Hort., *C. Forbesii* Lindl., *C. Mossiae* Hook., and *C. Trianae* Linden and Reichb. f. The condition has been known to orchid growers in California for some time and has always been associated with excessive watering, lack of ventilation, and low temperature.

Diseased seedlings growing in community pots damp off. A black discoloration appears in the growing point and the seedlings soon dry up completely (Fig. 1). The course of the disease is rapid and losses may be con-

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[1] The authors wish to express their gratitude to Mr. W. S. Sibray of the Alameda County Agricultural Commissioner's Office for assistance in the studies presented in this paper.

[2] Rosetti, Victoria. Podridão preta das Orquideas. O Biologico 9: 201–205. 1943.

[3] Limber, D. P. A note on the distribution of black rot of orchids. U. S. Dept. Agr., Plant Dis. Rptr. 30: 89. 1946.

siderable.   On the leaves of small individual plants, black spots of various
sizes (Fig. 2, A) may coalesce and form larger, black, dead areas.   In
plants 2 or 3 years of age the disease may start in the root and spread to-
ward the pseudobulb.   The attacked roots die, and the pseudobulbs shrivel
and finally lose all their contents.   The disease frequently occurs after the
plants are moved from one pot to another or when too much water is used.
It also may start at the leaf tip and spread rapidly downward when atmos-
pheric humidity is high (Fig. 2, E).   In large plants a black, shiny discol-
oration appears on the leaf blade.   Sometimes the tissues become soft and
flaccid and on slight pressure water exudes in abundance.   At this stage
the leaves are somewhat translucent.   In more advanced stages the tissues
collapse and the translucency disappears.   The pseudobulb between the
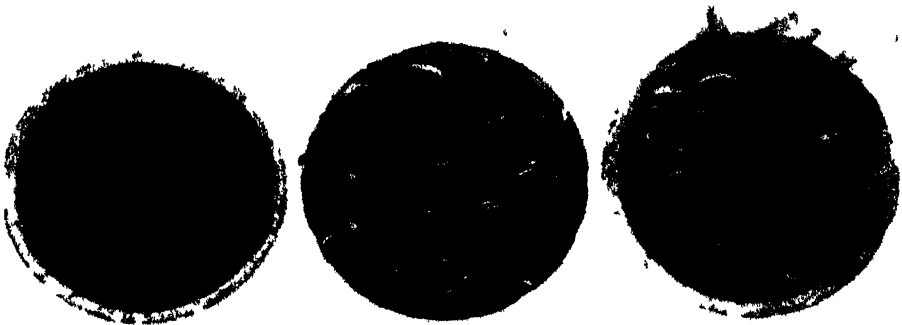
FIG. 1.   Damping-off of Cattleya seedlings caused by *Pythium ultimum*.   Severe
damage in pot on the left.

crown and the leaf blade may become infected through either of these
routes.   Light-yellow to light-brown discolorations, in the form of streaks
which enlarge longitudinally as well as transversely, soon change the pseu-
dobulb to a black mass.   The pathogen can spread from one pseudobulb
to another until the whole plant loses its leaves and becomes a mass of mum-
mified pseudobulbs.

Cultures of the pathogen were secured from damped-off Cattleya seed-
lings.   Plants with black discoloration in the crown were pulled and the
dead roots were cut off.   Samples were washed in 6 changes of sterile dis-
tilled water, then placed on sterile paper in sterile Petri dishes in order that
excess moisture might be absorbed.   Subsequently the samples were cut
into smaller pieces and transferred to acidulated potato-dextrose agar in a
Petri dish.   As soon as clean hyphae were seen under a dissecting micro-

scope, a piece of mycelium was transferred to potato-dextrose agar. In culturing the fungus from the pseudobulbs, these were split along the long
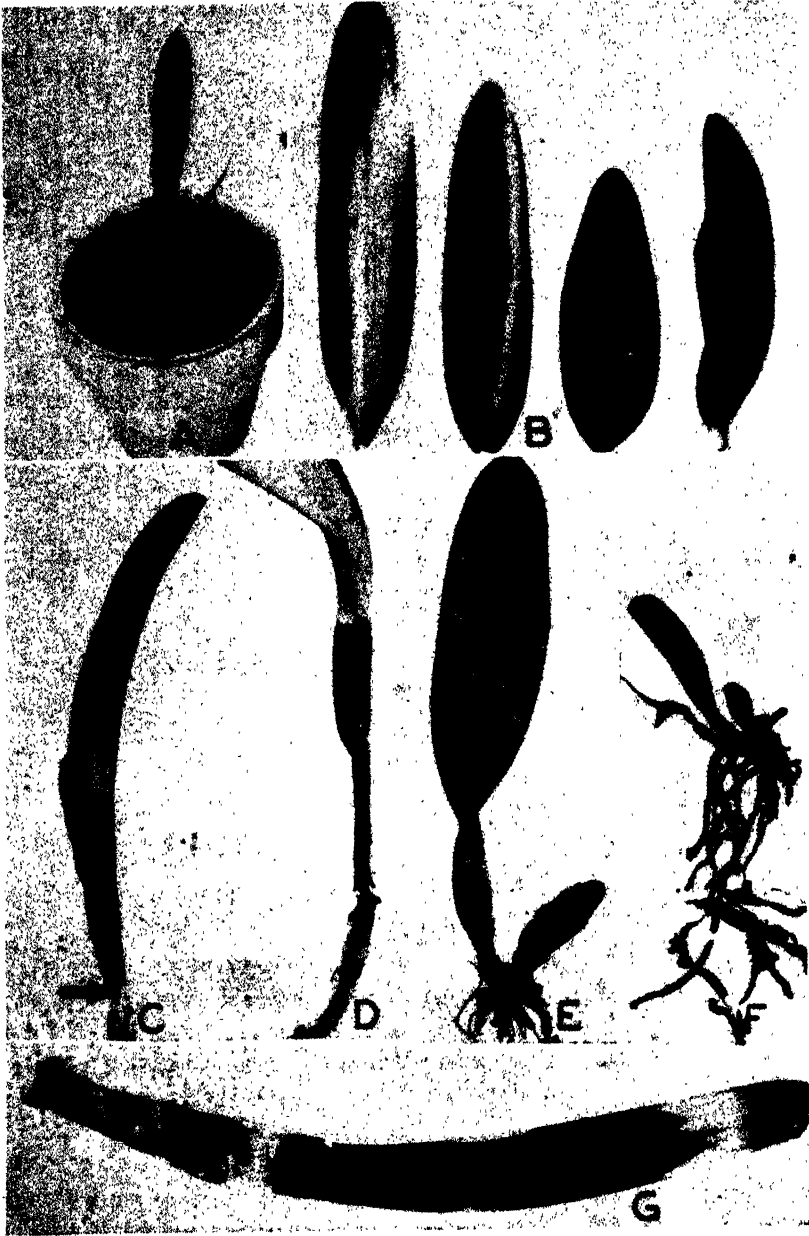
FIG. 2. Symptoms of Pythium black rot of orchid. A. Infections caused by zoospores of *Pythium ultimum* on leaf of *Cattleya Mossiae*. B. Infection on leaves of young Cattleya plants. The leaf on the right was soft and watery. C and D. Black rot produced by placing agar culture of the fungus on a pseudobulb. E. Infection of leaf tip and roots of *Cattleya* sp. from a commercial greenhouse. F. Severe black rot in a 5-year-old plant. G. Pseudobulb of *Cattleya Trianae* infected with *P. ultimum*.

axis and pieces of infected tissues were transferred, first to potato-dextrose agar acidified with lactic acid, and later to ordinary potato-dextrose agar. Leaf-blade tissues at the advancing margin of a lesion were used in the same way.

The fungus isolated conforms with *Pythium ultimum* Trow as defined by the junior author.[4]

Vegetative growth is abundant and arachnoid on media rich in carbohydrates. Fruiting structures are rarely produced on potato-dextrose agar. Reproductive bodies are produced in abundance on cornmeal agar and on plain water agar from potato-dextrose-agar blocks containing the fungus. Sporangia are mostly acrogenous on short, lateral branches, and measure 13.2 to 29.6 μ, average 21.9 μ, in diameter; intercalary sporangia 14.2 to 18.6 μ in width and 21.1 to 29.6μ in length occasionally occur.

The sporangia of *Pythium ultimum* commonly germinate by the production of germ tubes. Those of the orchid isolates were observed to germinate by the production of zoospores in a vesicle borne on a short emission tube. Although this manner of germination is rather unusual for sporangia in *P. ultimum*, it is not unique and cannot be considered a phenomenon alien to this species. Oogonia are similar to sporangia in disposition, shape, and size, and can only be distinguished from sporangia by the attendant antheridia and the oospores. Antheridia are monoclinous, and originate near the apex of the oogonial stalk; they are customarily sessile, short, inflated, and sharply upcurved, making apical contact with the basal portion of the oogonium. A fertilization tube is usually conspicuously present. The oospores are aplerotic, measure 19.4 to 23.9 μ, average 19.8 μ, in diameter, and are smooth and thick-walled, usually containing a single reserve globule and a subspherical refringent body.

Cardinal temperatures for vegetative growth of the orchid isolate are: minimum, 1° C.; optimum, 28° to 31° C.; maximum, 37° C. These values agree with those reported for *Pythium ultimum*.

To test the pathogenicity of the fungus, it was grown on potato-dextrose agar and small pieces were transferred to young pseudobulbs of *Cattleya Trianae*. These were kept at high humidity and a temperature of 65° to 75° F. Disease symptoms appeared after two days and the disease progressed rapidly (Fig. 2, C and D). *Pythium ultimum* was reisolated from infected plants. In some cases *P. ultimum* placed in a sterile watch glass with sterile water produced abundant zoospores; when these were sprayed on an unfolded leaf of a young plant, black spots developed on the leaf 5 to 8 days after spraying. This suggests the mode of spread of the fungus in orchid houses where overhead irrigation is practiced.

Inoculations of *Cattleya Fabia, C. Forbesii, C. Mossiae,* and *C. Trianae* resulted in typical symptoms. *Epidendrum O'Brienianum* Rolfe inocu-

[4] Middleton, John T. The taxonomy, host range, and geographic distribution of the genus Pythium. Mem. Torr. Bot. Club 20: 1–171. 1943.

lated with *Pythium ultimum* developed the disease. *Odontoglossum grande* Lindl. and *O. Schlieperianum* Reichb. f. failed to become diseased when inoculated with *P. ultimum.*

The seedling phase of the disease was controlled by watering the pots with a 1 : 2000 concentration of 8-hydroxyquinolin benzoate (Bioquin 700). The treatment can safely be repeated every week until no more disease appears. Watering the plants with a copper solution, 1 : 100,000 or 1 : 1,000,-000, was also effective in stopping the disease. This treatment can be repeated if necessary but not often, because too much copper may accumulate in the Osmunda fiber. Control of the disease on large plants should be centered around reducing the humidity of the air, keeping the plants dry, and providing plenty of ventilation. Sometimes it may be necessary to expose diseased plants to the sun.—PETER A. ARK and JOHN T. MIDDLETON, Division of Plant Pathology, University of California, Berkeley and Riverside, California.

Resistance in Alfalfa to Yellow Leaf Blotch. Resistance in alfalfa to yellow leaf blotch caused by *Pseudopeziza jonesii* (Fckl.) Nannf. has been observed in the nursery in two groups of selected plants. The first noted were two clons among four derived from survivors selected in 1944 from a nursery row seeded with alfalfa, Conference Number A 81. These clons first attracted attention because of resistance to *Pseudopeziza medicaginis* (Lib.) Sacc. The second group of plants were among selections from a field of Kansas common alfalfa. This field, seeded in 1947, attracted attention in the autumn of that year because it furnished an unusual opportunity to select plants resistant to *Pseudopeziza medicaginis.* The growth of this ample but not overly dense stand had been restricted by summer drought, and the autumn growth bore common leaf spot in great abundance mixed with only a little of the leaf spot caused by *Pleospora herbarum* (Pers. ex Fr.) Rab. Twenty-one plants with clean foliage were obtained from this field. The frequency of such plants was estimated to be of the order of hundredths of one per cent.

Not all of these selections proved in greenhouse tests to be very highly resistant to leaf spot, and a few were so poor in type that they were of little interest. However, in the spring of 1949 fourteen of these plants were represented in the nursery by clons and by small populations obtained by selfing. Leaf spot did not develop in the first growth in this nursery until very late, and even then in very small amount; but yellow leaf blotch did develop early in moderate amount, apparently from a single short period of infection. In a few clons that appeared to be especially susceptible to injury from yellow leaf blotch the lower leaves had fallen by the middle of June. Among the fourteen clons and selfed progenies resistant to leaf spot, four retained lower leaves almost free from yellow leaf blotch, as late as June 23. On June 29 well-developed blotch lesions were found on lower leaves of two of the four clons. The nursery was cut shortly after this.

Thus it appears that in at least two of the Kansas selections there is a type of resistance to yellow leaf blotch similar to a type of resistance to leaf spot in which young leaves are not infected readily or abundantly, but in which the fungus may survive and produce at least a few lesions in old leaves if they are retained on the plant.

Unfortunately it does not appear possible to test this apparent resistance immediately by controlled artificial inoculations. Suitable fruiting cultures have not been produced, but the use of apothecia collected in the field remains to be explored. Even with these handicaps, it appears highly desirable to seek new selections whenever opportunity offers, especially in wilt-resistant stocks.—FRED REUEL JONES, U. S. Department of Agriculture, Madison, Wisconsin.

A Mass-Production Method for Studies of Barley Smuts in the Greenhouse.[1]—With a view to utilizing greenhouse space as efficiently as possible in studies of the barley smuts, the writer has developed methods whereby from 30,000 to over 60,000 barley plants can be grown each year during the winter months in a greenhouse bench area of 420 sq. ft. About 8,000 to 10,000 plants would be grown by former methods.

Seeding directly in greenhouse benches often results in thin irregular stands, sometimes as low as 50 to 75 per cent, due to poor seed germination or to smut infection of emerging seedlings. To overcome this, H. A. Rodenhiser, one of the writer's associates, devised the method of seeding in soil in 4 × 8-in. tin pans, 2 in. deep. The pans are placed in covered tin boxes to check evaporation during seed germination. Moistened blotters usually are put in the tin boxes to maintain the humidity. The seedlings are transplanted to greenhouse benches when they are about 1 in. high. This method of germinating seed in chambers or rooms with controlled temperatures optimum for infection is now generally used by cereal pathologists at the Plant Industry Station, Beltsville, Maryland. The accurate control over temperature during the critical period of seed germination and the efficient use of greenhouse space resulting from full stands of plants fully justify the additional work involved.

Another step toward economy resulted from the discovery that under usual greenhouse conditions, small plants with a single culm 12 to 18 in. high served as well as much taller plants with numerous culms for indicating smut resistance or susceptibility.[2, 3] Barley plants of this size can be grown from spacing as close as 1 in. in rows only 4 in. apart. One hundred per cent smutted heads frequently has been obtained in the 4-in. plantings.

[1] The barley smuts involved were the covered smut (*Ustilago hordei* (Pers.) Lagerh.), the nuda loose smut (*U. nuda* (Jens.) Rostr.), and the nigra loose smut (*U. nigra* Tapke).

[2] Reed, G. M. Influence of growth of the host on smut development. Proc. Amer. Phil. Soc. 79: 303–326. 1938.

[3] Tapke, V. F. Influence of varietal resistance, sap acidity, and certain environmental factors on the occurrence of loose smut in wheat. Jour. Agr. Res. [U.S.] 39(5): 813–339. 1929.

Spacings 6 to 8 in. between rows formerly were used. Plant growth is restricted also by light watering two or three times a week instead of daily. In one experiment barley plants were grown 1 in. apart in rows only 2 in. apart. Up to 83 per cent smutted heads were obtained on susceptible varieties. The writer would hesitate to recommend this spacing without qualification but the experiment indicates the possibility of very thick planting.

Economy also resulted from the practice used by J. Allen Clark of growing two crops of wheat per year instead of the usual one crop in the greenhouse at Beltsville, Maryland. The first crop was sown September 10. From mid-October until maturity in November or December the plants received artificial light from 5 P. M. to 11 P. M. daily. Four 100-watt mazda bulbs under reflectors were used for a bench 17¾ by 4¾ feet. The bulbs were spaced equally over the long axis of the bench and hung approximately 1 ft. above the top of the plants. A second crop of wheat sown in January matured in March or April without supplemental artificial light. The writer has used this two-crop system during the past 3 years. It works very well with spring barleys grown from smutted seed and it doubles the capacity of the greenhouse space.

On the basis of their studies on photoperiodicity, Borthwick et al.[4] suggested to the writer that he might effect economy without loss of efficiency by using daily supplemental lighting from 12 midnight to 2 A. M. instead of from 5 P. M. to 11 P. M. During the 2 years that the writer has tried this the plant growth and the incidence of smut have been satisfactory.

Some studies of small-grain smuts require a great amount of tedious work in processing the seed. When the test is conducted in the field, the hazard of conditions unfavorable to infection is a constant menace and it may be necessary to repeat the processing several times or more before conditions satisfactory for good infection are obtained. When the seed is sown under the controlled conditions of a greenhouse, however, the success of the test is practically certain. This seems to add incalculably to the value of a mass-production method of greenhouse testing.—V. F. TAPKE, Senior Pathologist, Division of Cereal Crops and Diseases, U. S. Department of Agriculture, Plant Industry Station, Beltsville, Maryland.

[4] Borthwick, H. A., S. B. Hendricks, and M. W. Parker. Action spectrum for photoperiodic control of floral initiation of a long-day plant, Wintex barley (*Hordeum vulgare*). Bot. Gaz. 110: 103–118. 1948.

INDEX FOR VOLUME 39

ERRATA, VOLUME 38

Page 874, text line 12, *read* 1½ pints oil *for* 1½ pints fish oil
Page 925, line 19, *delete* and a part of a third
Page 1021, table 2, test 4, *read*:

Untreated	60	73
Wrapped, untreated paper	60	87

for:

Untreated		
Wrapped, untreated paper	60	73
Wraps, containing 1.5 per cent (etc.)	60	87

Page 1034, line 30, *read* (× *Fragaria ananassa* Duch) *for* (of × *Fragaria ananassa* Duch.)

ERRATA, VOLUME 39

Pages 43 and 45, runninghead: *read* virulence *for* virulance
Page 79, line 14, *read* two dent corn hybrids *for* two, dent corn hybrids
Page 271, line 5, *read* 1948 *for* 1946
Page 392, line 12, *read* protoplasmic *for* photoplasmic
Page 415, line 12, *read* Box 87 *for* Box 28
Page 529, footnote 2, line 2, *read* M J. Johnson *for* M. S Johnson
Page 535, table 5, column 1, *read* 0.125 *for* 1 125
Page 652, figure 2 replaced by:

Page 825, line 10, *read* ml *for* liter
Page 840, lines 37 and 38, *read* (*Melilotus alba* Desr.) *for* (*Melilotus officinalis* (L.) Lam.)
Page 840, line 40, *read* (*M. officinalis* (L.) Lam.) *for* (*M. alba* Desr.)
Page 841, line 22, and page 842, line 1, *read* When the virus was recovered, although no symptoms were observed, the degree of susceptibility was designated as "latent."